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THE UNIVERSITY OF ALBERTA

STUDIES ON THE MICROBIAL AND CHEMICAL MODIFICATION OF SOYBEANS FOR HUMAN CONSUMPTION

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled

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submitted by David J. Schroder in partial fulfilment of the requirements for the degree of Master of Science.

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ABSTRACT

The object of this research study was the production of an inexpensive palatable soybean product with good storage qualities. A review of the literature has been made on the effects of heat treatments, soaking and processing techniques which greatly influence the beany flavors, growth inhibitors, nutritive value and overall acceptability of soybean products. A review of Oriental and western utilization of soybeans for human consumption is presented.

Factors affecting the production, storage and acceptability of a soybean cheese using a lactic starter organism (*Streptococcus thermophilus*) and rennet with skimmilk powder blended with soybean milk in the proportions of 25%, 50% and 75% of the total dry weight were studied. The effects of surface ripening of soybean cheeses using *Rhizopus oligosporus*, *Penicillium camemberti* and a tempeh extract were also evaluated. It was shown that the use of microorganisms and enzyme systems for the modification and utilization of soybeans, although bringing about desirable changes in texture, results in the formation of undesirable bitter flavors.

In order to develop a more acceptable product, it was necessary to immediately inactivate the lipoxidase enzyme by blending the whole beans in water at 80-100°C, followed by filtration and precipitation of the curd with calcium sulfate.

Nutritional studies and an amino acid analysis were carried out to assess the nutritional value of this product. Methionine appears to be the limiting factor in the biological utilization of the protein.

The approximate cost of the product would be about $ .09 per pound, based on the cost of raw materials.

Synthetic meat products made from the soybean curd by the addition of emulsifiers and flavoring compounds were found to be very acceptable by a taste panel.

In conclusion, this study has shown that it is possible to prepare a soybean milk almost free of a beany flavor. Precipitation of the bland soybean milk with calcium sulfate yields a curd that can be easily flavored and packaged to yield a range of very acceptable, inexpensive and nutritious synthetic meat products.

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INTRODUCTION

The Soybean

The soybean (*Glycine max*) is one of the oldest crops cultivated by man. The first recorded history of the soybean plant in China was written in Sheng Nang's "Materia Medica" in 2838 B.C. (Lo *et al*. 1968). Soybean protein is unique among vegetable proteins by virtue of its relatively high biological value. This oil-rich legume has received extensive attention all over the world as a source of protein for human diets. It has been prepared in a variety of forms for human consumption and the literature is voluminous (Circle and Johnson 1958, Markley 1950-51). Although animal products are the more desirable protein sources, they are more expensive to produce than plant proteins. This is due to the comparative efficiency of the yield of edible protein produced per acre. Developments in this field are of special interest at this time because of the ever increasing demand for protein brought about by the rapid increase in the world population.

Soybean is one of the most nutritive crops known. It contains approximately 40% protein, 20% fat and 20% carbohydrate. It

is a good source of calcium, vitamins A, B and D (Reid, 1943). Hackler _et al_.(1963, 1967) demonstrated the nutritional value of various soybean protein fractions on growing rats. Dutra de Oliviera _et al_. (1966, 1967) showed in a comparative study that soybean milk compared favorably with cow's milk in the nutrition of malnourished children.

Table I shows the essential amino acid composition of soybeans compared to some animal products and the Food and Agriculture Organization Pattern.

TABLE I

Essential Amino Acid Composition of Some Food Proteins

Amino Acid	FAO Pattern	Whole Egg	Cow's Milk	Beef Muscle	Wheat	Soybean
	Mg Amino Acid per G Nitrogen					
Isoleucine	278	388	341	323	253	319
Leucine	305	564	620	488	409	483
Lysine	270	383	475	537	174	429
Methionine and cystine	275	344	214	253	265	197
Phenylalanine and tyrosine	360	574	599	428	457	557
Threonine	180	309	280	276	192	269
Tryptophan	90	71	81	63	67	80
Valine	270	437	409	321	272	336

From Altschul, 1965

The FAO Pattern was developed by the Food and Agriculture Organization to give a provisional pattern of the average minimum essential amino acid requirements of adults and infants for each individual amino acid (Oldham, 1961). The soybean compares very favorably with the FAO Pattern except for the sulfur-containing amino acids, methionine and cystine. Supplementation of soybean protein diets with methione results in a more efficient biological utilization (Smith, 1961).

The net protein value of a food in nutrition will depend on the amino acid content, the digestibility and the biological value (Mitchell, 1950). Table II shows the net protein value (on a moisture-free basis) for a growing rat of optimally heated soybeans and soybean flour compared with other high protein foods.

TABLE II

Net Protein Value of Soybean Products in Comparison with Other High-Protein Foods*

Food product	Protein content, %	True digest-ibility,%	Digestible protein content,%	Biological value,%	Net protein content,%
Eggs	49.2	100	49	94	46
Beef, round	58.5	100	59	76	44
Soybean flour, medium fat	46.7	96	45	75	34
Cheese, cheddar	39.2	98	38	72	28
Soybeans, optimally heated	37.7	96	36	75	27
Milk, whole	26.7	95	25	90	23

* All results expressed on a moisture-free basis.

From Mitchell, 1950

Soybeans in their whole unmodified state are relatively indigestible due to the presence of antidigestive and growth depressant factors. Soybeans also have a peculiar and undesirable beany flavor that is not easily removed. They must, therefore, be processed before being consumed.

In examining the Oriental pattern of soybean usage, which has been an important form of dietary protein for centuries, in nearly all cases the soybeans are either fractionated or modified by fermentation, generally with molds, but sometimes with bacteria, yeasts or mixtures of microorganisms. Western peoples are not familiar with these foods, although they are eaten by millions of people and constitute some of

the most common foods on the earth. Western processing includes removal of the oil, heating the residues to drive off undesirable substances and developing meals, flours, finely pulverized milk substitutes and texturized products that closely resemble meats. Looking at it from an economic point of view, the Oriental methods are relatively cheap and simple while the cost of the modern processed imitation animal products are high.

Processing of Soybeans

The initial procedure in the manufacture of most soybean products is the preparation of a soybean milk. The way in which the milk is produced will significantly affect the nature of the final product. Soybean milk has been prepared in the Orient by a standard method of soaking the soybeans in water, followed by grinding in water, filtering and cooking for about 30 minutes. The effects of presoaking, dehulling and heat treatment will be considered in detail because of their marked effects on the efficiency of processing and the nutritive value and acceptability of the finished product.

i) Presoaking

Although presoaking is a standard practice in the Oriental methods for utilization of soybeans, there are two major disadvantages to this procedure. The first of these is the loss of soluble solids in the soak water. This is shown in Figure 1 (Wilkens *et al*. 1967). Lo *et al*. (1968) found that as the soaking time for soybeans increased, larger quantities of water soluble solids leached into the soak water where they were lost in the usual methods of manufacturing soybean milk. Changes, apparently metabolic, in the soybeans during soaking caused

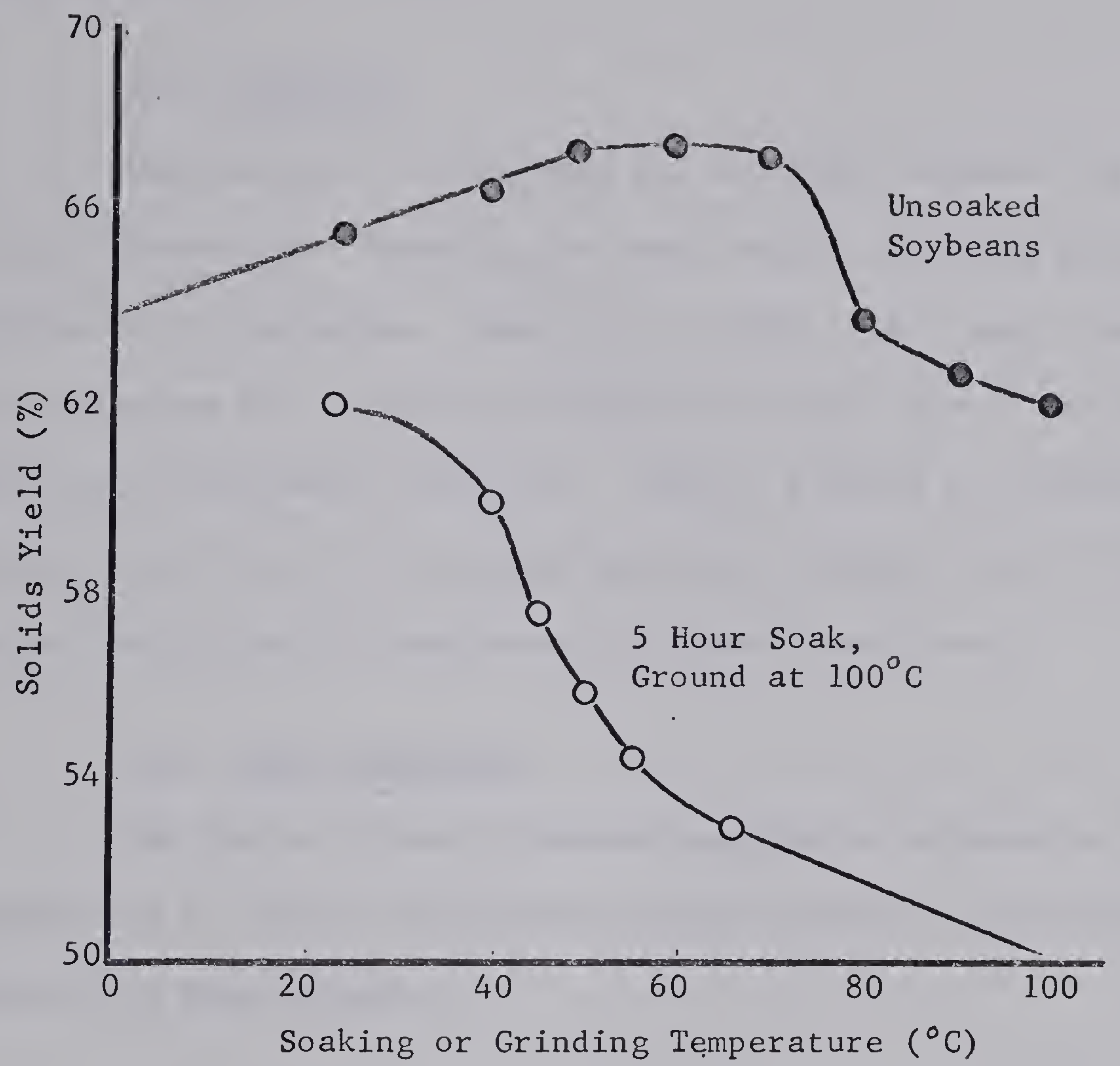


Figure 1. Effect of hydration and grinding temperatures on the solids yield.
From Wilkins et al. 1967

protein to decrease from 43% in the non-soaked control to 33% for the 24 hour soak (1°C) to 36% for the 72 hour soak. Non-protein nitrogen increased during the same intervals from 0.16% to 0.28% at 24 hours and to 0.86% at 72 hour soak. The increase in non-protein nitrogen materials presumably consisted of polypeptides and amino acids, the breakdown products of protein. There was also a decrease in the fat content from 24% (non-soaked control) to 19% (72 hour soak).

The second disadvantage of presoaking is the activation of lipoxidase, an enzyme known to be associated with the production of undesirable flavor compounds. This will be covered more fully in a

later section.

ii) Dehulling

The removal of hulls from dry untreated soybeans is a difficult procedure. Steaming the beans before dehulling greatly facilitates the operation. Hand *et al*. (1964) found that treatment with live steam for 45 minutes loosened the hulls and at the same time inactivated the growth inhibitors. Shorter periods of steaming, 2 - 3 minutes, were found to be equally effective, however, such treatments were not sufficient to inactivate the growth inhibitors.

iii) Heat Treatment

The degree of heat treatment applied to soybeans is very important as it affects the flavor, growth inhibitors and nutritive value of the final product.

a) Flavor

The flavor of soybeans has been recognized as a major problem in the preparation of foods for human consumption. The most undesirable flavors have been described as "raw bean" flavor, "green bean-like" flavor and bitter. In view of the importance of the flavor principles, much work has been done to identify the active components.

Arai *et al*. (1966) fractionated an ethanol extract of defatted soybean flour and analysed the phenolic acid fraction thought to be the main flavor causative agent in soy flour. The fraction had a strong phenol flavor and contained at least seven phenolic acids including syringic, vanillic, ferulic, gestisic, salicylic, p-coumaric and p-hydroxybenzoic acids. The main component was syringic acid. In addition, two isomers of chlorogenic acids having sour, bitter and

astringent flavors were found.

The major objectionable flavor has been described as "green bean-like". This flavor is not present in the original intact, raw, whole soybean but develops immediately after maceration of the bean or after the beans have been presoaked.

According to Wilkens _et_ _al_.(1967), the "soybean off-flavor" is mainly associated with volatile compounds due to the oxidation of the polyunsaturated fats catalysed by lipoxidase enzymes. Pre-soaking of the beans apparently brought the lipoxidase system to a potentially more active state at the time of grinding.

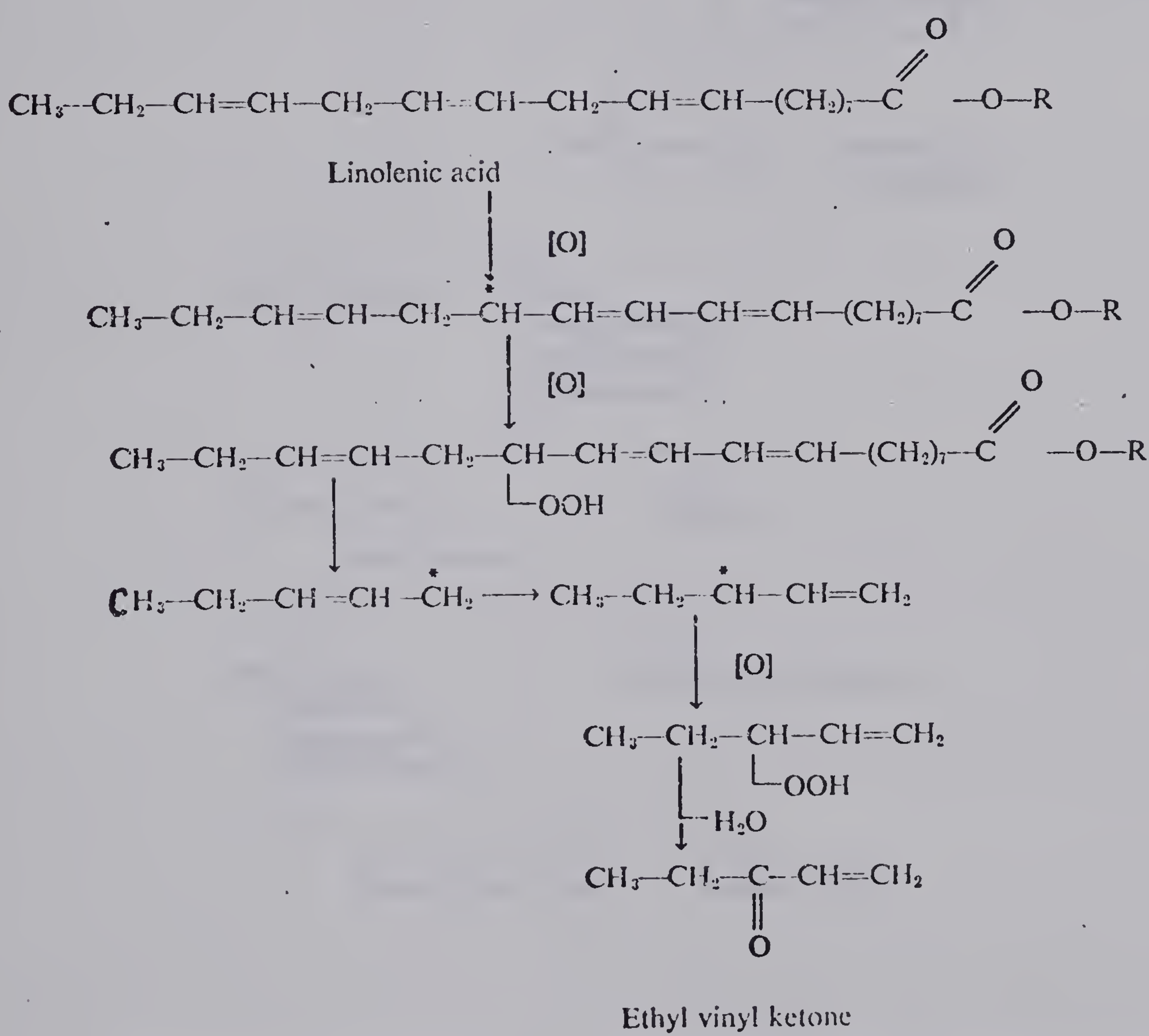
Wilkens _et_ _al_.(1967) found that an acceptable bland milk was produced by grinding soaked dehulled soybeans with water at temperatures between 80-90°C and maintaining this temperature for 10 minutes to completely inactivate the lipoxidase enzyme.

Fujimaki _et_ _al_.(1968) stated that the existence of undesirable flavor components in defatted soybean flour and soybean protein showed some interaction between the flavor components and the soybean protein thereby preventing removal of the components by solvent extraction. Fujimaki _et_ _al_. proposed that the application of proteolytic enzymes to soybean protein, which through breakdown of the interaction by partial hydrolysis of the protein in mild conditions, could remove the flavor. In evaluation by sensory tests, he found the beany flavor in the early stage of digestion was removed but very quickly bitter flavors were produced. These bitter flavors which are probably derived from peptides are a general phenomenon encountered in protein hydrolysis.

Mattick and Hand (1969) carried out an extensive and elaborate study of the "beany" flavor of soybeans. Using gas chromato-

graphy and mass spectrometry, they isolated and identified ethyl vinyl ketone as the major beany principle. This finding confirmed the suggestions of Hill and Hammond (1965) who attributed the off-flavors produced in the early stages of autoxidation of soybean oil to mixtures of pentanal and ethyl vinyl ketone.

The theories of autoxidation of unsaturated fatty acids, together with the action of lipoxidase in soybean milk, could advance a possible mechanism for the formation of "beany" flavors by the oxidation of linolenic acid (Mattick and Hand, 1969):



Most recently Honig _et al_.(1969) made a detailed study of the flavor components of free and bound lipids of dehulled and defatted soybean flakes. The lipids were fractionated and characterized by

chemical and chromatographic procedures. The procedure for fractionation is shown in Figure 2 and the characterization of the fractions is summarized in Table III. Peaks I to VI are the flavor characteristics of soybean lipid column fractions obtained by silicic acid chromatography.

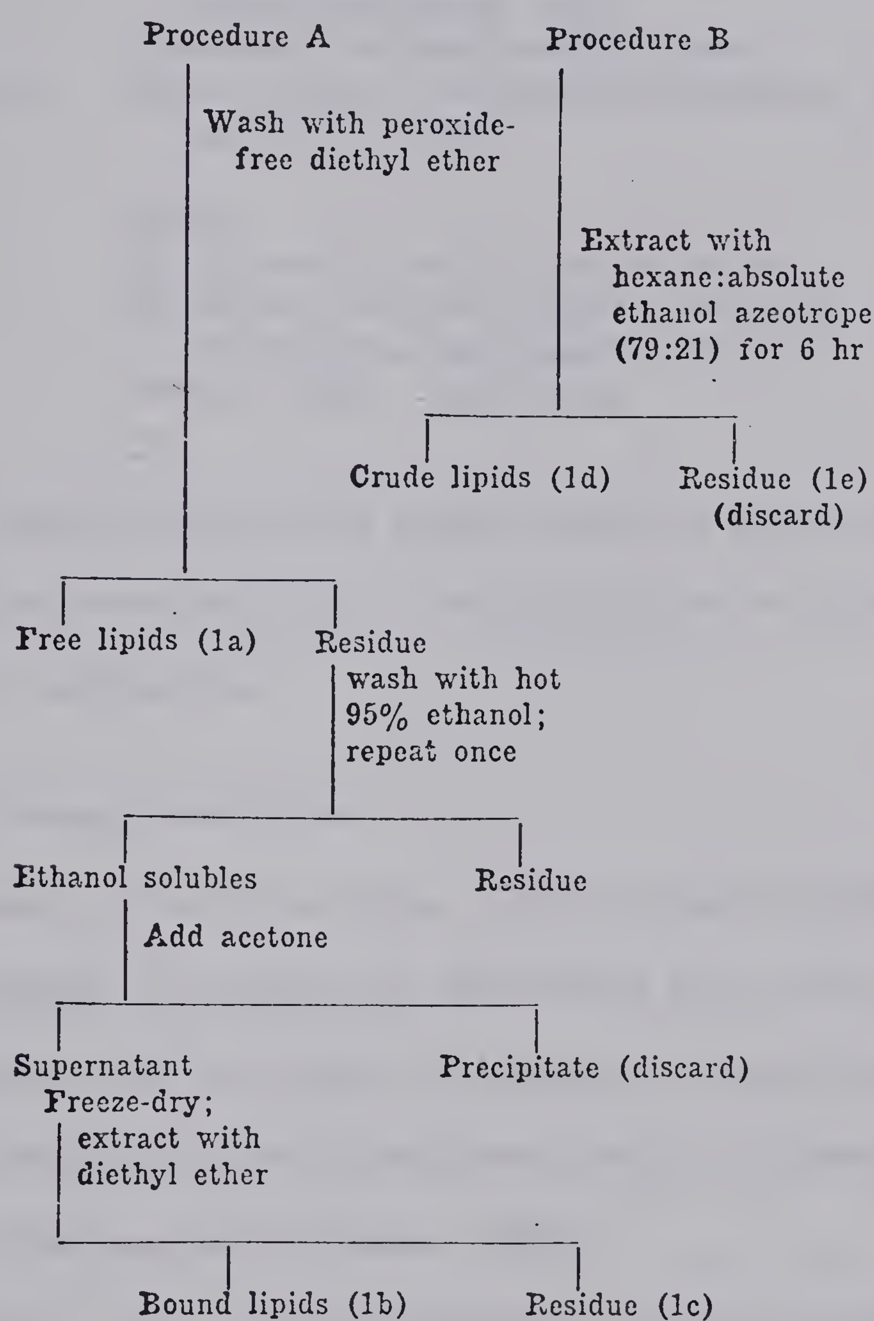


Figure 2. Extraction of the lipids from dehulled, defatted soybean flakes.

Table III

Flavor Characterization of Various Soybean Meal Fractions

Fraction	Flavor
Raw defatted soybean meal	Green, beany, bitter, a throat-catching, lingering aftertaste
Free lipids (1a)	Oily, waxy
Bound lipids (1b)	Hydrocarbon, lingering aftertaste, biting, throat-catching, oily
Residue (1c)	Intensely bitter, mealy, sweet
Crude lipids (1d)	Hydrocarbon, lingering aftertaste, biting, throat-catching, oily
Azeotrope residue (1e)	Bland
Peak I	Oily, waxy, painty, hydrocarbon
Peak II	Molasses, bitter, throat-catching, lingering aftertaste, astringent, biting
Peaks III-VI	Mealy, waxy, shortening

It is apparent that the beany flavor of soybeans is a very complicated system associated with the degradation of proteins and lipids and their interaction.

b) Growth inhibitors

Soybeans in their raw state contain growth inhibitors. Proper heat treatment is capable of destroying the toxic or undesirable factors in soybeans such as trypsin inhibitors, hemaglutinin, saponin, a goitrogenic substance, an anticoagulant factor, a diuretic principle, and lipoxidase (Pomeranz and Lindner, 1960).

In general, the growth inhibitors are more sensitive to heat than are the major proteins of the soybean. Therefore, heat will destroy these inhibitors preferentially and thus, improve the nutritive value of the soybean. Figure 3 (Klose *et al.*, 1948) shows the effect of the type and extent of heat treatment for the destruction of growth inhibitors and the increase of the nutritional value.

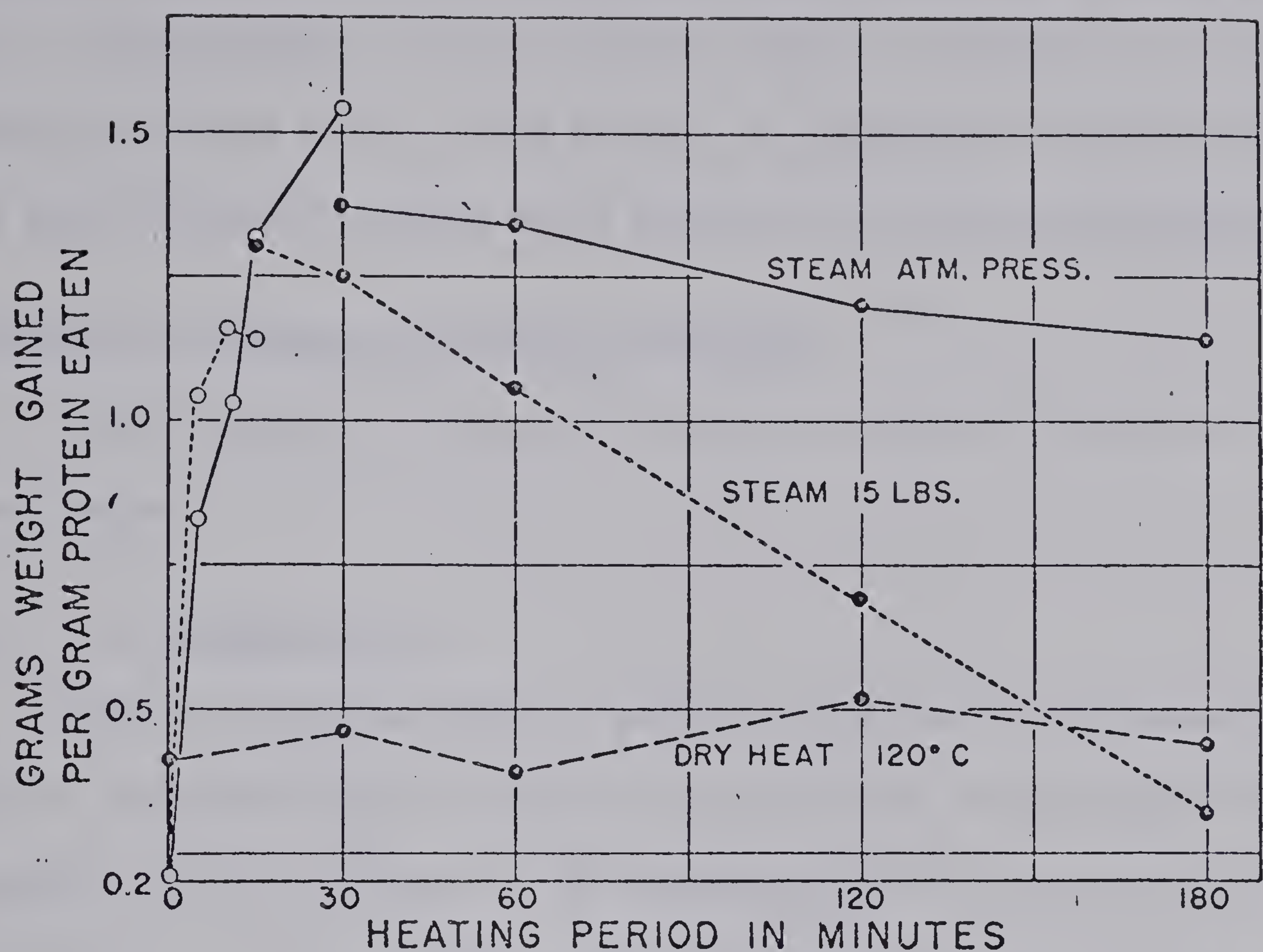


FIG. 3. Effect of type and extent of heat treatment on nutritional value of soybean protein.[1]

Experiment 1 — Experiment 2

———— Steam at atmospheric pressure

- - - - - - Steam at 15 lb. pressure

— — — — Dry heat 120° C.

[1] Test period, 42 days; 12 rats per group; 80 grams average initial weight.

From Klose et al. 1948

c) Nutritive value

Excessive heat treatments can denature the protein and decrease its nutritional value as well as its extractibility in water (Altschul, 1958 and Wilkens et al., 1967). The improvement in the nutritional quality of soybean protein resulting from mild heat treatment is due partly to the destruction of the growth inhibitors and partly to the modification of the protein permitting more complete digestibility and utilization of the growth-limiting sulfur amino acids (Longenecker et al., 1964).

The effect of overheating on certain nutritional properties

of protein of soybean (120°C for 2 hours) was studied by Iriarte *et al.* (1966). Nutritionally, the only factors found to contribute to the decreased nutritive value of the protein of excessively heated soybean were a destruction of cystine and a decrease in nitrogen absorbability.

Utilization of Soybeans for Human Consumption

The literature on the utilization of soybean products is reviewed below.

i) Soybean Milk

The term soybean milk is generally applied to the aqueous extract of soybeans which is similar to cow's milk in appearance but the flavor is quite different. The association of the term "milk" in the description of this soybean extract is unfortunate as it may be misleading with respect to cow's milk. It is, however, in common usage and will be followed to prevent confusion.

Soybean milk has been prepared in the Orient by a standard method of soaking beans in water for several hours followed by grinding with water, filtration and cooking for about 30 minutes. Soybean milk, with some added sugar, is drunk by the Chinese in the early morning and is also eaten as a broth with salted pickles.

Hand *et al.* (1964) developed a newer method for the factory production of powdered soybean milk. They stated that a dry soybean milk can be made directly from whole dehulled soybeans without including the water extraction steps. The hulls were loosened by steaming for 45 minutes. The beans were dried and then dehulled by passing through a burr mill refiner to crack the cotyledons. The hulls were separated from the cotyledons by passing them over a

gravity separator. The beans were then ground and slurried in warm water and passed through a homogenizer and then spray dried. Soybean milk is manufactured by modern techniques and distributed as a beverage in Hong Kong, Singapore, Manila, Taiwan, etc., and on a dietetic and medical basis in the western countries (Abbott, 1965).

ii) Soybean Curd (Tofu)

Soybean curd or "Tofu" is made by precipitating the protein from the milk previously described by means of acids or salts (Burnett, 1951). After the curd settles, the supernatant liquid is removed and the curd is placed on cloths and spread in trays which serve as molds. Sufficient water is removed by pressing until the curd is solid enough to be handled. The curd has the consistancy of cream cheese and is cut into small squares and sold. The fresh tofu contains 80-90% water, 5-8% protein, 3-4% fat and about 2-4% carbohydrate. Because of the high water content, the curd is subject to spoilage and is generally prepared and consumed each day.

iii) Soybean Cheese

Due to the rapid spoilage of soybean curd or tofu, attempts have been made to preserve the product by using microorganisms. Wai (1929) described a process for the production of a Chinese soybean cheese or "Sufu". First, the tofu was cut into blocks, arranged on bamboo trays and left in the fermentation chamber for a month. The average temperature of the fermentation was 14°C. After this treatment, the blocks are put into large earthenware barrels and salt and Shoushing wine added. The barrels are closed and left for three months. Corville (1929) reported that Chinese soybean cheese was made by fermentation

of tofu with a species of _Mucor sufu_.

Different species of fungi isolated from soybean cheese have been reported by Hesseltine (1965). They all belong to the family _Mucoraceae_. The role of fungi is to excrete enzymes that breakdown the soybean protein to peptides and amino acids. The mold should be dense and thick enough to form a solid film over the surface of the tofu. The mold should not impart a disagreeable odor or taste.

Hang and Jackson (1967a) developed a method for preparing a soybean cheese under controlled conditions using a lactic starter organism _Streptococcus thermophilus_. The protein was precipitated from soybean milk by acid produced by the organism. The resulting curd was handled and stored by conventional cheese making procedures. Further research (Hang and Jackson, 1967b) on soybean cheese was carried out by adding starter organisms, rennet extract and skimmed cow's milk to the soymilk. The addition of skimmilk and rennet extract reduced the protein coagulation time and improved the flavor of the finished product.

iv) _Soy Sauce_

Soy sauce, which is consumed throughout the world, is a dark brown salty liquid made by the fermentation of soybeans and wheat. According to Minor (1945), soy sauce consists of a mixture of amino acids, peptides, polypeptides, peptones, simple proteins, purines and lesser amounts of other organic compounds suspended in an 18% salt solution.

Soy sauce is prepared using specific types of molds such as _Aspergillus flavus_, _A. niger_ and _A. oryzae_. Each mold imparts a

distinctive flavor and character to the finished product. The process is as follows: two parts precooked beans are mixed with 1.5 parts crushed roasted wheat. The entire mass is inoculated with a mold culture such as _A. oryzae_. When the mold has developed sufficiently, the entire mass is placed in a salt solution and allowed to react in sterilized glass vessels at 120°F for 6-8 weeks. The sauce is then pressed out of the grain mass, filtered, pasteurized and bottled.

An alternate method of producing soy sauce is acid hydrolysis of soybeans.

v) _Miso_

Miso is a fermented food made from ground soybeans and rice or barley, with the addition of salt. It is widely used in Japan as a base for hot breakfast soup and as a flavoring agent with various foods, such as vegetables and meats (Burnett, 1951).

According to Shibasaki and Hesseltine (1961), the manufacture of miso is a process involving two separate and distinct fermentations. The first involves the aerobic pure culture fermentation of rice with selected strains of _A. oryzae_ to prepare koji (mold rice) as a source of enzymes and nutrients for the second fermentation - the fermentation of mold rice, salt and ground soybeans. In the second fermentation, the inoculum used is a suitable sample of good miso from an earlier fermentation.

Hesseltine and Shibasaki (1961) developed a new process of manufacturing miso with a pure culture of _Saccharomyces rouxii_. According to their results, a pure culture fermentation eliminates all the contaminating microorganisms introduced by using old miso as a

starter. Another advantage was that the fermentation, as judged by odor, always began much sooner in the pure culture fermentation than in the old method.

vi) Tempeh

Tempeh is a popular Indonesian food produced by fermenting cooked whole dehulled soybeans with a species of *Rhizopus* (Steinkraus *et al*., 1960). During fermentation, the soybeans become bound together into a compact cake by the mold mycelia. The soybean cake is then sliced, dried or roasted, cooked in soup or deep fat fried before being eaten. In the traditional method, the soybeans are wrapped in banana leaves for fermentation. Martinelli and Hesseltine (1964) developed a process to make tempeh rapidly in large amounts by pure culture fermentation with *R. oryzae* in shallow trays or plastic bags. Steinkraus *et al*. (1965) developed a pilot-plant process for the production of dehydrated tempeh, as this product may be a low cost product for use in world wide food programs.

vii) Western Products

In western countries the starting material for most soybean foods is dehulled, defatted soybean meal.

There are four main types of modern soy products available for use in foods (Anon., 1967). First, there is the soybean concentrate containing between 50-60% protein. Secondly, there is a full fat soy flour of 40% protein. The full fat flour and the 50-60% concentrate are used as food additives in baking, beverages, etc. A third soy product is the 70% soy protein concentrate. This product has the maximum nutritive value as all the undesirable growth inhibitors are

removed. This product is easily incorporated into textured protein products. The fourth modern soy product is the soy protein isolate of 95-100% protein. The third and fourth products offer a new dimension in the creation of new foods - the incorporation of protein concentrates into spun textured foods.

The spun textured protein is a special case of processed products from soybeans which deserves further mention due to its potential market use. The process is described by Kelly and Pressey (1966). Isolated protein can be extruded through fibre-forming equipment into long threads or fibres. To produce these filaments, the defatted soybean meal is extracted with dilute caustic to solubilize a maximum of protein. Here, the globular soybean protein is converted to unfolded polypeptide chains and new sulfide bonds are formed. Since the extract also contains carbohydrates, pigments and other organic matter, the protein fraction is isolated by isoelectric precipitation at pH 4.5. After washing and diluting, the acid-precipitated protein is redissolved in a strong alkaline solution to yield a viscous preparation called a "dope" solution. This "dope" solution is extruded through spinnerettes into an acid-salt coagulation bath forming the fibres. The acidification brings the many polypeptide chains together favoring hydrogen and ionic bonding. By proper controlled conditions, any texture or aspect of chewiness can be accomplished. After being washed, these fibres are ready to be processed into food products.

Due to the fact that spun protein fibres are white in color and have a very bland taste, colors and flavors can be added to make a particular food product (Thulin and Kuramoto, 1967). The current applications of spun protein fibres and soybean protein isolate are

(Smith and Wolf, 1961, Harper, 1965):

1. nutritional supplements in selected food products,
2. used in the modification of certain physical characteristics of various products, such as aerating agents, water binding, emulsifying properties, etc., and
3. in the simulation of particular meats and meat products.

In 1966, there were over twenty different variations of synthetic beef, pork, fish and poultry products available in the United States. Most of these products are sold in health food stores (Deering, 1966). The economics of production is the limiting factor in the manufacture of spun protein products.

From this introduction, it is apparent that soybeans could make a significant contribution to the human diet, particularly with respect to protein. The major limitations to the consumption of soybeans at the present time are palatability and the cost of production.

The object of this research study was to produce an inexpensive, palatable soybean product with good storage qualities. The initial approach was essentially a continuation of the work by Hang and Jackson (1967a and b).

EXPERIMENTAL

Preparation of Soybean Milk

Preliminary experiments were carried out on the whole raw soybeans to assess the value of various steam treatments for facilitating removal of hulls and for the destruction of growth inhibitors and removal of the beany flavors. The following method was adopted. The process is summarized in Figure 4.

Soybean Milk Production

Whole soybeans
↓ wash
Steam 3 min
↓ dry 10 min at 105 °C
Crack hulls and remove
↓ add H_2O (ratio 9:1 at 80-100 °C)
Blend 3 min
↓
Steam 30 min
↓
Soybean milk

Figure 4. Flow sheet for the production of soybean milk.

Dry mature washed soybeans (Grade No. 1, W.G. Thompson and Son, Limited, Blenheim, Ontario, Canada) were steamed 3 minutes at atmospheric pressure to loosen the hulls. Surface moisture was removed by drying for 10 minutes at 105°C. The beans were cracked in a properly spaced cereal grinder (Model 4-E Quaker City Mill, The Straub Company, Philadelphia, Pa.) and the hulls were removed by air separation.

The cotyledons were placed in a Waring Blender, tap water at 80-100°C was added in the ratio of 9:1, and the mixture was blended for 5 minutes. The resulting suspension was steamed for 30 minutes to destroy any remaining growth inhibitors. The resulting liquid was used for the preparation of the starter and soybean curd.

Preparation of Soybean Cheese Using Starter

i) Starter Organism

A freeze-dried culture of *Streptococcus thermophilus* 101 (Klenzade Products, Division of Economics Laboratory, Inc., Beloit, Wisconsin) was used in the present study.

ii) Development of Starter

100 mg of freeze-dried culture was added to a flask containing 100 g of autoclaved soybean milk (20 minutes at 15 psi and 121°C) and incubated at 37°C for 15 hours. One ml of this mother starter was added to 100 g of autoclaved soybean milk and after incubation, the process was repeated using 2.5 ml starter in 250 ml of milk. The resulting starter culture was stored at 4°C until required.

iii) Preparation of Soybean Cheese

Skimmilk powder was blended with soybean milk in the proportions of 25%, 50% and 75% of the total dry weight. Five kg of the soybean-skimmilk mixture was brought to 41°C and 5% of starter culture was added. The mixture was incubated at 41°C in a water bath. Rennet extract (Hansen's Cheese Rennet, Horan-Lally Co. Ltd., Rexdale, Ontario, Canada), 0.3% by volume, was added 1 hour after the starter. When fine lines of whey appeared where a knife had cut the jelly-like curd, the

curd was ready to be cut. After cutting, the curd was cooked by slowly raising the temperature of the water bath to 48°C (1°C per minute).

When cooking finished, the curd was placed into hoops and pressed at 1 psi for 24 hours. The cheeses were then waxed and stored at 20°C. The process is summarized in Figure 5.

Cheese Production

Soybean milk + Skimmilk in portions of 25%, 50% and 75% of the total dry weight

↓

Add starter (41°C)

↓ 1 hour

Add rennet extract (0.3%)

↓ 2 hours

Cut curd

↓ 15 min

Begin cook (1°C/min to 48°C)

↓ 30 min

End cook (48°C)

↓

Dip and hoop

↓

Press 24 hours

↓

Wax

↓

Store at 20°C

Figure 5. Flow sheet for the production of soybean cheese.

Four replicates of 25%, 50% and 75% skimmilk were prepared. Each replicate was called a "make". The purpose of the experiment was

to evaluate the effects of the addition of skimmilk powder to soybean milk in the quality of curd production and storage quality of the cheese produced. Data was collected during the manufacturing process of the number of starter organisms, the pH and the protein content. Analyses at weekly intervals were made on the pH, number of starter organisms, protein breakdown, moisture content, texture and product acceptibility.

iv) Mold Ripening

To evaluate flavor and texture changes associated with mold ripening, two experiments were carried out on soybean cheeses. The first experiment consisted of inoculating cheese surfaces with *Rhizopus oligosporus* and *Penicillium camemberti*. The second experiment was the production of a cheese from a water extract of soybean tempeh.

a) *Rhizopus oligosporus*

R. oligosporus spores were produced on Bacto Mycological Agar.

Bacto-Soytone	1.0%
Bacto-Dextrose	1.0%
Bacto-Agar	1.5%

The surface of a soybean cheese, 1 inch high and 5 inches in diameter, was smeared with mold mycelium of *R. oligosporus*. This was wrapped in rubber netting to allow a very small amount of air around the cheese surface. This was wrapped in aluminum foil and incubated at 38°C for 18 hours. The cheeses were then stored at 20°C for 3 weeks.

b) *Penicillium camemberti*

P. camemberti spores were produced on Czapek's Agar.

Sucrose	4.0 %
$MgSO_4$	0.05%
KH_2PO_4	0.10%
KCl	0.05%
$NaNO_3$	0.30%
Agar (Bacto)	1.50%

The surface of a soybean cheese, 1 inch high and 5 inches in diameter, was smeared with mold spores of _P. camemberti_ and allowed to dry at room temperature (22°C) for 5-6 hours. The cheeses were then hand dipped in coarse salt (NaCl). Any excess salt was shaken off and the cheeses left to dry overnight.

The dried cheeses were then transferred to a rack in a dessicator jar containing a solution of $Na_2HPO_4 \cdot 12H_2O$ to maintain the humidity at approximately 95%. The dessicator plus cheeses was incubated at 10°C for 14 days, the cheeses being turned after 5 days. After incubation, the cheeses were wrapped in aluminum foil and stored at 4°C.

c) Tempeh Extract

Tempeh was produced by the following method (Steinkraus _et al_., 1965):

Dehulled soybeans were soaked 30 minutes in dilute lactic acid at 100°C (3 ml 85% lactic acid/300 ml water/100 g beans). The pH of the beans was between 4.8 to 5.0. This prevents the growth of potential bacterial spoilage organisms. The mixture was boiled a further 90 minutes then drained and cooled to 38°C. The beans were then inoculated with mycelium of _R. oligosporus_, tightly packed in petri dishes, and incubated for 18 hours at 37.5°C.

The resulting tempeh cakes were ground in water at 90°C (9 parts water:1 part original dry beans) to produce an extract. The normal method was used to produce a cheese from this extract. The cheese was stored at 20°C for 3 weeks.

The mold ripened cheeses and the cheese produced from tempeh were compared to soybean cheese controls stored for 3 weeks at 20°C.

Preparation of Soybean Curd Using Calcium Sulfate

The method for producing soybean curd is outlined in Figure 6.

Soybean Curd Production

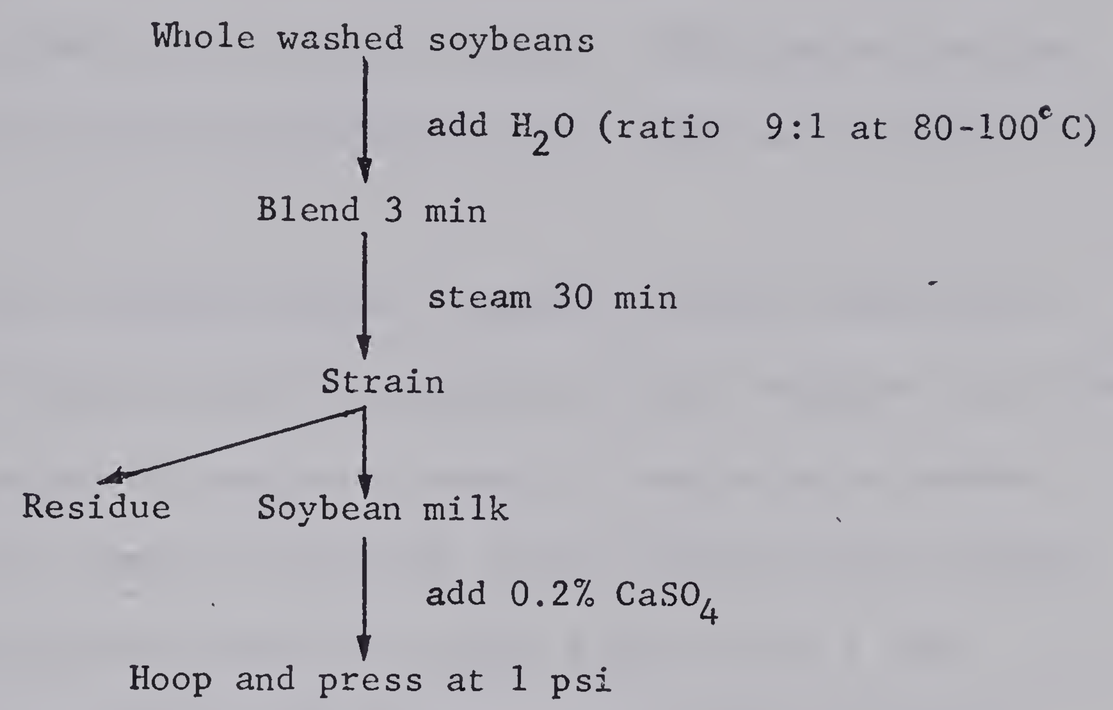


Figure 6. Flow sheet for the production of soybean curd.

Six soybean curd samples were produced. The pressed curd samples were waxed and stored at 20°C. Three samples were dry salted prior to waxing and the other three were kept as controls.

Sampling

The technique for sampling the batch contents for bacterial investigations and pH determinations during the manufacturing process was that developed by Frazier et al.(1934). A sterile 50 ml beaker was used for sampling. The curd was allowed to settle, the whey poured off into a sterile beaker and the curd transferred to a sterile mortar and ground for 1 minute. Approximately 0.2 g of sodium citrate was then added for each gram of curd and the grinding continued until a smooth even paste was formed. The decanted whey was poured in and the contents

of the mortar were thoroughly stirred until the resulting fluid was homogeneous. One ml of this fluid was used for making plate counts.

The samples were taken in the same manner for making pH determinations. The curd and whey were separated and the curd was ground without the addition of sodium citrate. The decanted whey was returned to the curd and pH determinations were made on the mixture of whey and curd.

During the ripening process, samples from the cheese were removed at weekly intervals for determination of pH, moisture, total and water-soluble nitrogen and bacterial numbers. Samples were removed with a sterile trier from the hoop side of the soybean cheese halfway between the top and bottom surfaces to give a 1½ inch by 1 inch diameter plug. The nature of the cheese was such that mixing was accomplished easily in a sterile beaker. Samples were taken from this mixture for analysis.

Determination of Bacterial Numbers

The medium used for the enumeration of bacteria in soybean cheese during the manufacture and ripening processes (a modification of media developed by Donovan and Vincent, 1955) had the following composition:

Medium

Agar	15 g
Bactotryptone	5 g
Yeast extract	3 g
Lactose	1 g
Bromo-cresol purple powder	0.05 g
Phenol red powder	0.12 g
Distilled water	to 1000 ml
Final pH	6.8

This is a double indicator media to determine acid and alkali producers. The method for determining the bacterial counts during ripening was modified from a method described by Burkey (1931). The method used was as follows: 1 g of cheese was ground for 1 minute in a sterile mortar with 3 ml of sterile Ringers solution (Oxoid Limited, London, England). Sodium citrate (0.2 g) was then added and the grinding continued until a smooth, even paste was formed. Sterile Ringers solution (6.5 ml) was then added and the mixture thoroughly agitated to give a 1:10 dilution of the cheese. Further serial dilutions were made for plating.

pH Measurement

The pH of the cheeses was measured by inserting the glass electrode of a Beckman Model 72 pH meter into a beaker containing a representative sample.

Total and Water Soluble Nitrogen Content

Total nitrogen was determined by the standard micro-Kjeldahl procedure (AOAC, 10th ed. 1965). Water soluble nitrogen was determined by an adaptation of the method of Smith *et al.* (1952). A finely ground sample was extracted with water at room temperature (20°C) on a reciprocating shaker (Eberbach Corporation, Ann Arbor, Michigan) at 60 r.p.m. for 1 hour. The suspension was centrifuged for 6 minutes at 2,000 x g and the sample of the supernatant was analysed by the micro-Kjeldahl procedure.

Moisture Content

Moisture content was determined by drying samples to constant weight in a vacuum oven at 100°C.

Determination of Hardness

Hardness of soybean cheese was measured by means of a Precision penetrometer (Precision Scientific Company, Chicago, Illinois).

Taste Panel Evaluation

To evaluate the removal of the "beany" and other objectionable flavors as a result of the processing method, a taste panel was set up. The curd produced was compared for flavor and texture with a sample of Chinese soybean curd produced by the traditional method.

The taste panel form is shown in Figure 7. Three samples were given together to a total of 23 untrained taste panel members. On the first two samples (the test sample and tofu or Chinese soybean curd), the panel was requested to give the extent of the "beany" flavor and some indication of the texture. All three samples (two test and one Chinese soybean curd) were given to the panel to rank in order of preference and to determine if members of the panel could pick out which sample was different.

Amino Acid Analysis

Amino acid analysis on the isolated proteins was carried out in the Beckman Spinco model 120-B amino acid analyzer (Beckman Instruments Inc., Fullerton, California) according to Spackman *et al.* (1958). Hydrolysis of the protein was effected in 6N HCl at 110°C for 24 hours and corrections were made for hydrolytic losses by extrapolation of values back to zero hydrolysis time. All results are reported as percentage of total hydrolysate, however, no measurement of tryptophane was conducted.

Evaluation of Soybean Curd

I Check appropriate space

A) "Beany" flavor

	Sample I	Sample II
None		
Very Slight		
Moderate		
Pronounced		
Very Pronounced		

Other flavor - please comment

B) Texture - Check more than one if applicable

	Sample I	Sample II
Firm		
Chewy		
Rubbery		
Mealy		
Crumbly		
Spongy		
Smooth		
Pasty		

Other - please comment

II Place in order of preference

Sample I ________________

Sample II ________________

Sample III________________

III Comments:

Figure 7. Soybean curd evaluation form.

Determination of Crude Fat in Soybean Products

The crude fat was determined according to the official method for crude fat determination in soy flour (Cereal Laboratory Methods, 1962).

Determination of Gross Energy of Soybean Curd

The gross energy of the soybean curd and the test diet were determined in a Parr oxygen bomb calorimeter (Parr Instrument Company, Moline, Illinois).

Nutritional Studies

Forty weanling Sprague-Dawley rats of the University of Alberta strain, equalized with respect to sex and randomized with respect to litter origin, were allocated to two dietary groups at an average age of 21 days and an average weight of 50.2 g. The control diet contained casein as the sole source of protein while the test diet contained soybean curd as the sole source of protein. The soybean curd was freeze-dried in a RePP, 42-FFD-WS Sublimator (The Virtis Company Inc., Gardiner, New York) and ground to a fine powder for use in this trial. The rats were housed individually in stainless steel cages, 7 inches wide, 10 inches deep, 7 inches high. The cages were in banks in an air conditioned room maintained at 23°C and a relative humidity of 45-50%. The diets were fed *ad libitum* and water available at all times.

The formulation and composition of the experimental diets are given in Table IV. The diets were formulated to meet or exceed the minimal nutrient requirements for growth of the weanling rat as set down by the National Academy of Sciences - National Research

Table IV

Formulation and Composition of Diets Used to Evaluate the Nutritional Value of Soybean Curd

Ingredients	Control %	Test %
Sucrose	48.88	43.38
Casein[1]	25.00	
Soybean curd		42.50
Cellulose	5.00	5.00
Corn oil	15.00	3.00
Mineral mix[2]	5.00	5.00
Fat soluble vitamin mix[3]	0.50	0.50
Water soluble vitamin mix[4]	0.50	0.50
Choline chloride	0.12	0.12
Composition (by analysis)		
Crude protein (N x 6.25)%	22.94	22.31
Gross energy kcals/g	4.91	4.67

1. Vitamin free.
2. Composition, g/100 g mix: $CaCO_3$ 30.0; KH_2PO_4 34.1; NaCl (iodized) 25.0; $MgSO_4 \cdot 7H_2O$ 10.0; $FeCl_2 \cdot 4H_2O$ 0.60; $CuSO_4 \cdot 5H_2O$ 0.157; $MnSO_4 \cdot H_2O$ 0.12; $ZnCl_2$ 0.02; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ 0.003.
3. Composition, g/100 g mix: Vitamin A (10,000 IU/g) 20.0; Vitamin D_2 (35,000 IU/g) 4.0; Myvamix (20,000 IU Vitamin E/lb) 20.0; Corn starch 56.0.
4. Composition, g/100 g mix: Thiamine HCl 0.20; Riboflavin 0.20; Niacin 1.00; Calcium pantothenate 0.80; Pyridoxine HCl 0.10; Vitamin B_{12} 0.001; Biotin 0.004; Vitamin K (menadione) 0.02; Sucrose 97.675.

Council (United States)(1962). The diets were further formulated to be isonitrogenous and isocaloric. Formulation was based on analysis of the major ingredients. Nitrogen was determined by the Kjeldahl method and gross energy determined with the aid of a Parr oxygen bomb calorimeter.

The rats were fed the experimental diets for a period of 28 days during which time they were weighed at weekly intervals and their feed consumption recorded. At the time of weekly weighings, wasted

feed was collected, recorded, and the feed consumption corrected for wastage. Average daily gain, average daily feed, feed conversion (g fed/g gain) and the protein efficiency ratio (PER) were calculated. The data was analysed statistically by the analysis of variance as described by Steel and Torrie (1960).

Synthetic Meat Products

The soybean curd produced by precipitation with calcium sulfate mixed with varying amounts of spices and other ingredients according to suggestions by Frank and Circle (1959). The soybean curd was ground and formulated in the following proportions:

Soybean curd	468 g
$Na_3PO_4 \cdot 12H_2O$	12 g
Vegetable protein hydrolysate	1 g
Emulsifier	0.5 g
Spices	Dependant on
Smoke flavor	type or
Color	brand used

The mixture was stuffed into frankfurter casings and steamed for 25 minutes. In following tests, the mixture was sealed in number 307x113 cans (Continental Can Company of Canada) and autoclaved for 30 minutes.

Synthetic Meat Evaluation

Thirty untrained taste panel members were individually presented with a sample of canned synthetic meat flavored soybean curd. The members were allowed to taste the sample with or without bread and butter if they so desired. Their acceptance of the sample was rated on a scale ranging from very acceptable to very unacceptable (Figure 8).

Flavored Curd Evaluation

Very acceptable ______

Moderately acceptable ______

Neither like nor dislike ______

Moderately unacceptable ______

Very unacceptable ______

Comments:

Figure 8. Rating scale for the evaluation of meat flavored soybean curd.

RESULTS

Soybean Cheese Prepared Using Starter Cultures

i) Characteristics Immediately after Manufacture of the Soybean Cheese Prepared Using Soybean and 25%, 50% and 75% Skimmilk

The characteristics of the cheese are shown in Table V.

Table V

Chemical Analysis of the Cheeses Following Manufacture

	25% Soybean (75% Skim)	50% Soybean (50% Skim)	75% Soybean (25% Skim)
% protein (D.W. basis) (N x 6.25)	79.95	65.4	58.6
H_2O soluble N	0.21	0.14	0.11
Moisture	65.76	69.08	71.33
Penetrometer*	178	181	203
pH	4.64	4.59	4.52
% yield	51.6	64.8	74.4

* The higher the value, the softer the cheese.

It can be seen that there is an increase in the percentage protein, water soluble nitrogen and pH with an increasing concentration of skimmilk. Similarly, there is an increase in moisture content, yield and softness with increasing concentrations of soybean. These differences will be considered in more detail in the next section on changes occurring during ripening of the soybean-skimmilk cheeses.

ii) Changes Occurring During Manufacture and Ripening of Soybean-Skimmilk Cheeses

a) Bacterial numbers

Viable plate counts were made at the time of addition of starter, at cutting, immediately before dipping and at weekly intervals for nine weeks. The results are shown graphically in Figures 9 to 15. Each curve represents the results of duplicate analysis. Detailed results are tabulated in Appendix A. All organisms found in the cheeses were acid producing cocci (starter bacteria) with the exception of a few molds. This was determined by the indicator media used and by microscopic examination.

The viable count of the starter bacteria in the cheese shows some fluctuations but in general, the numbers increased greatly during the manufacturing period, dropped off during pressing and then, in general, remained at that level. There is little difference between the 25%, 50% and 75% soybean cheeses.

b) pH measurement

pH determinations were made on the soybean milk, at cutting, immediately before dipping and at weekly intervals for nine weeks. The results are shown graphically in Figures 16 to 22. Each curve represents the results of duplicate analysis. Detailed results are tabulated in Appendix B. The pH dropped rapidly during the manufacturing process and more gradually during storage. The cheeses containing the larger amounts of soybean were more acidic. This could be due to the greater buffering capacity of the skimmilk protein.

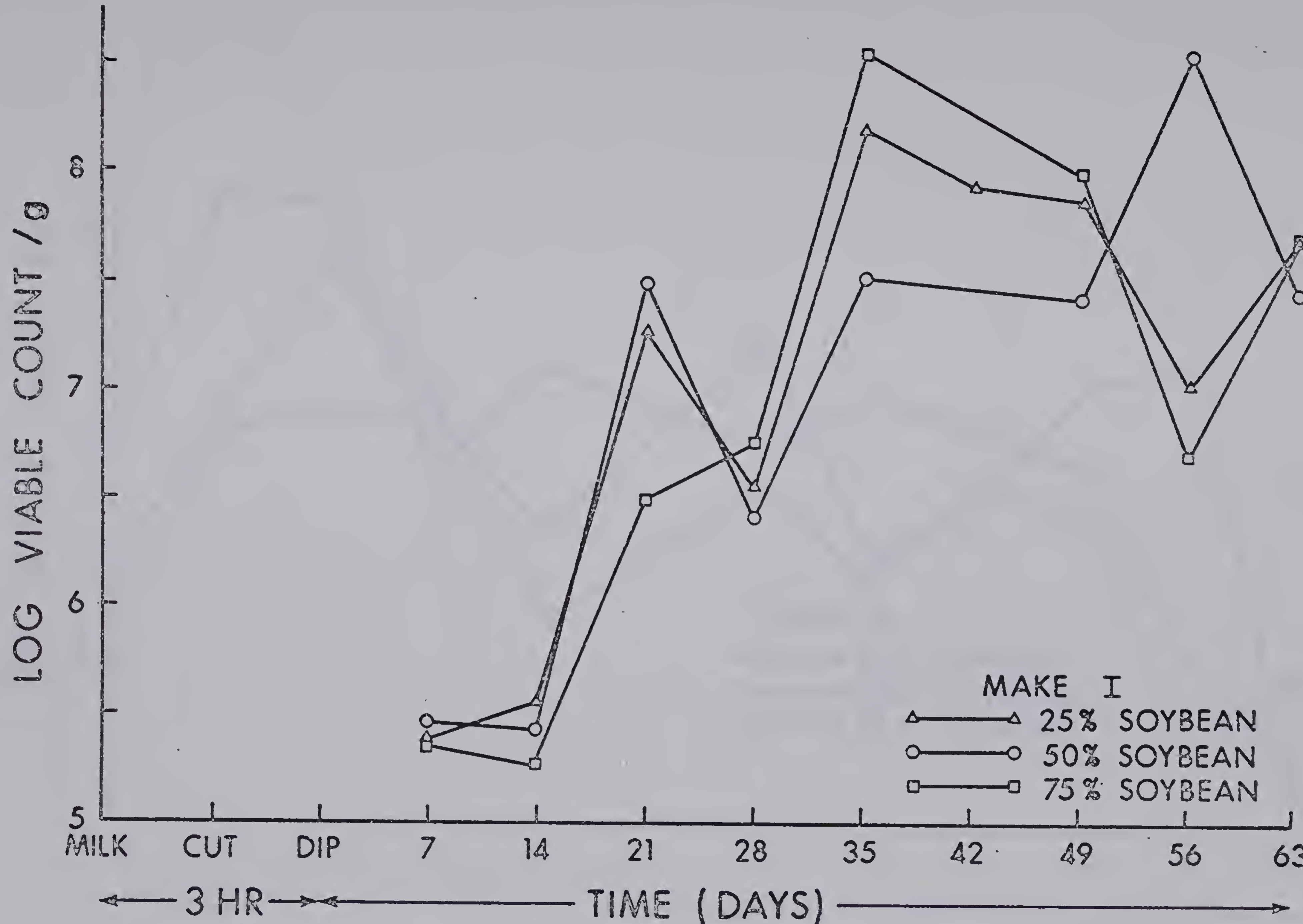


FIGURE 9: CHANGES IN BACTERIAL NUMBERS DURING MANUFACTURE AND RIPENING OF SOYBEAN-SKIMMILK CHEESES.

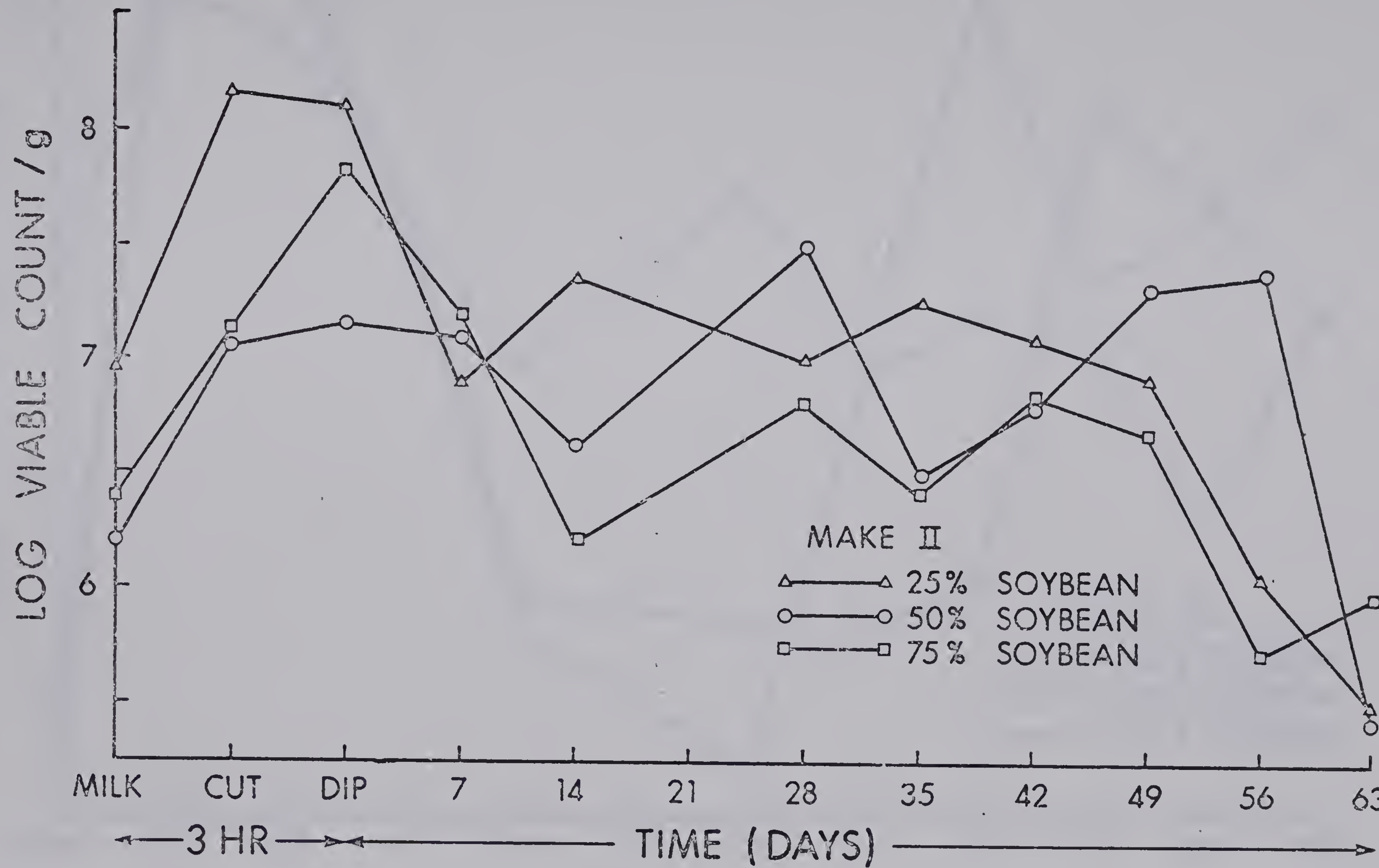


FIGURE 10: CHANGES IN BACTERIAL NUMBERS DURING MANUFACTURE AND RIPENING OF SOYBEAN-SKIMMILK CHEESES.

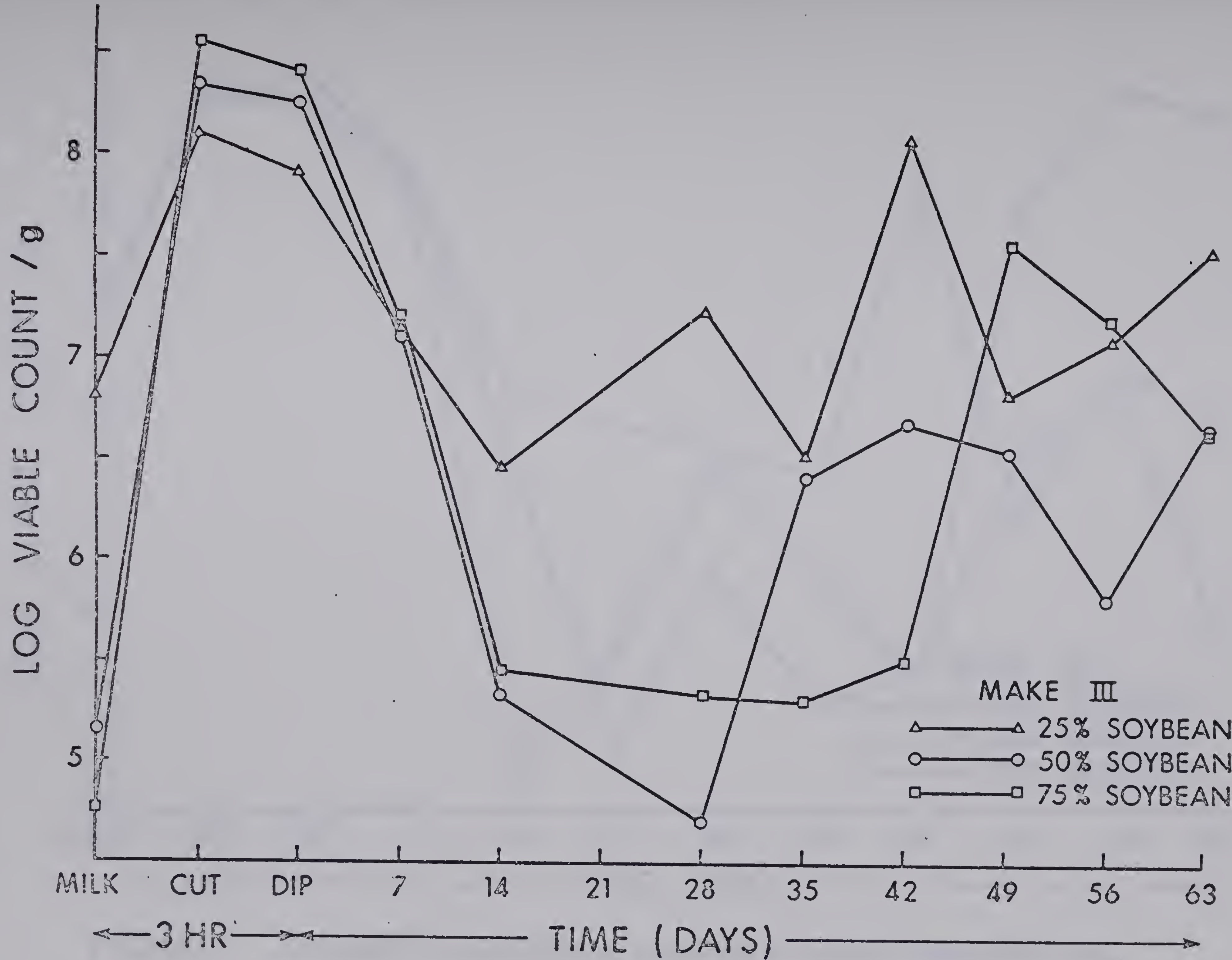


FIGURE 11: CHANGES IN BACTERIAL NUMBERS DURING MANUFACTURE AND RIPENING OF SOYBEAN-SKIMMILK CHEESES.

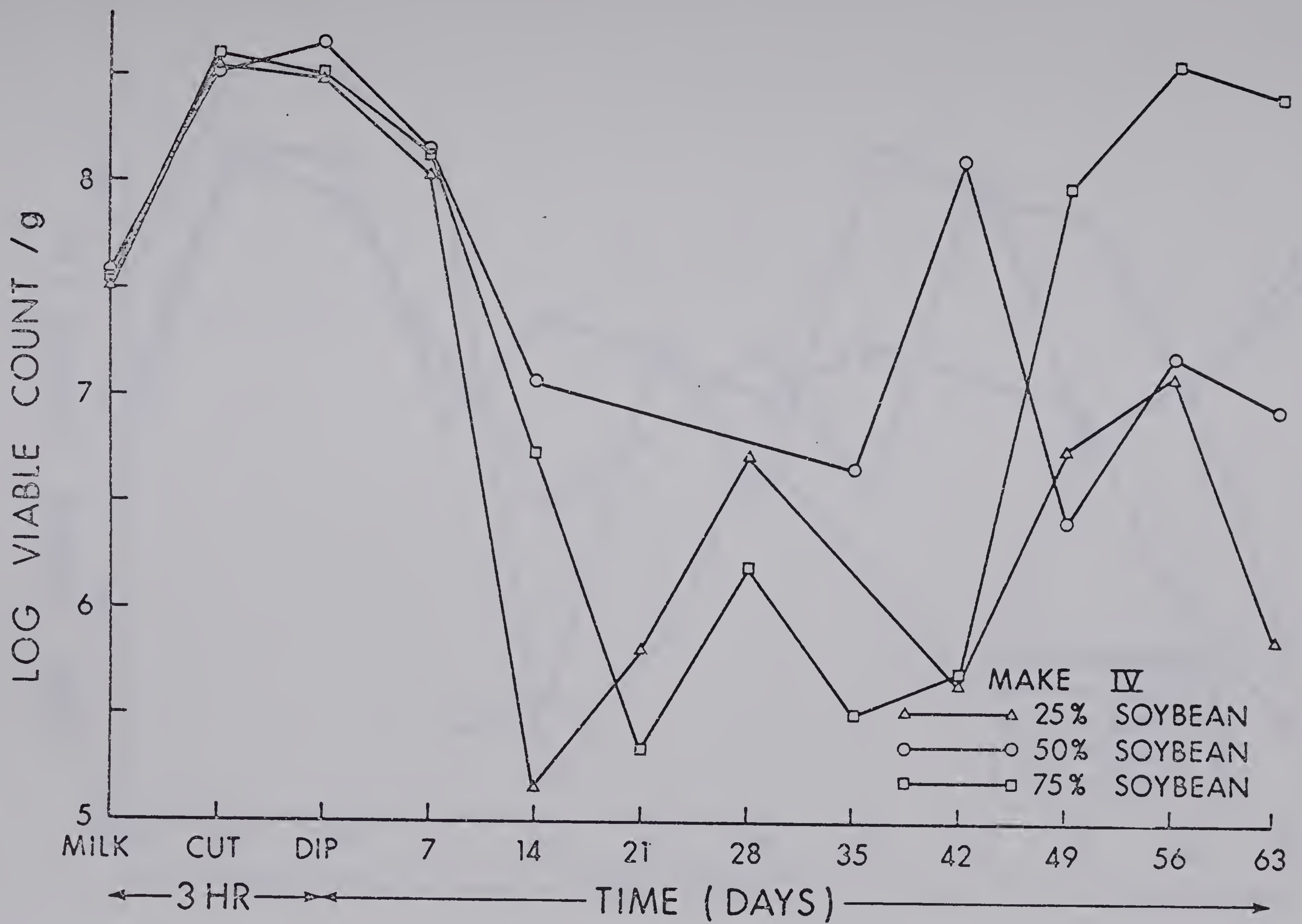


FIGURE 12: CHANGES IN BACTERIAL NUMBERS DURING MANUFACTURE AND RIPENING OF SOYBEAN-SKIMMILK CHEESES.

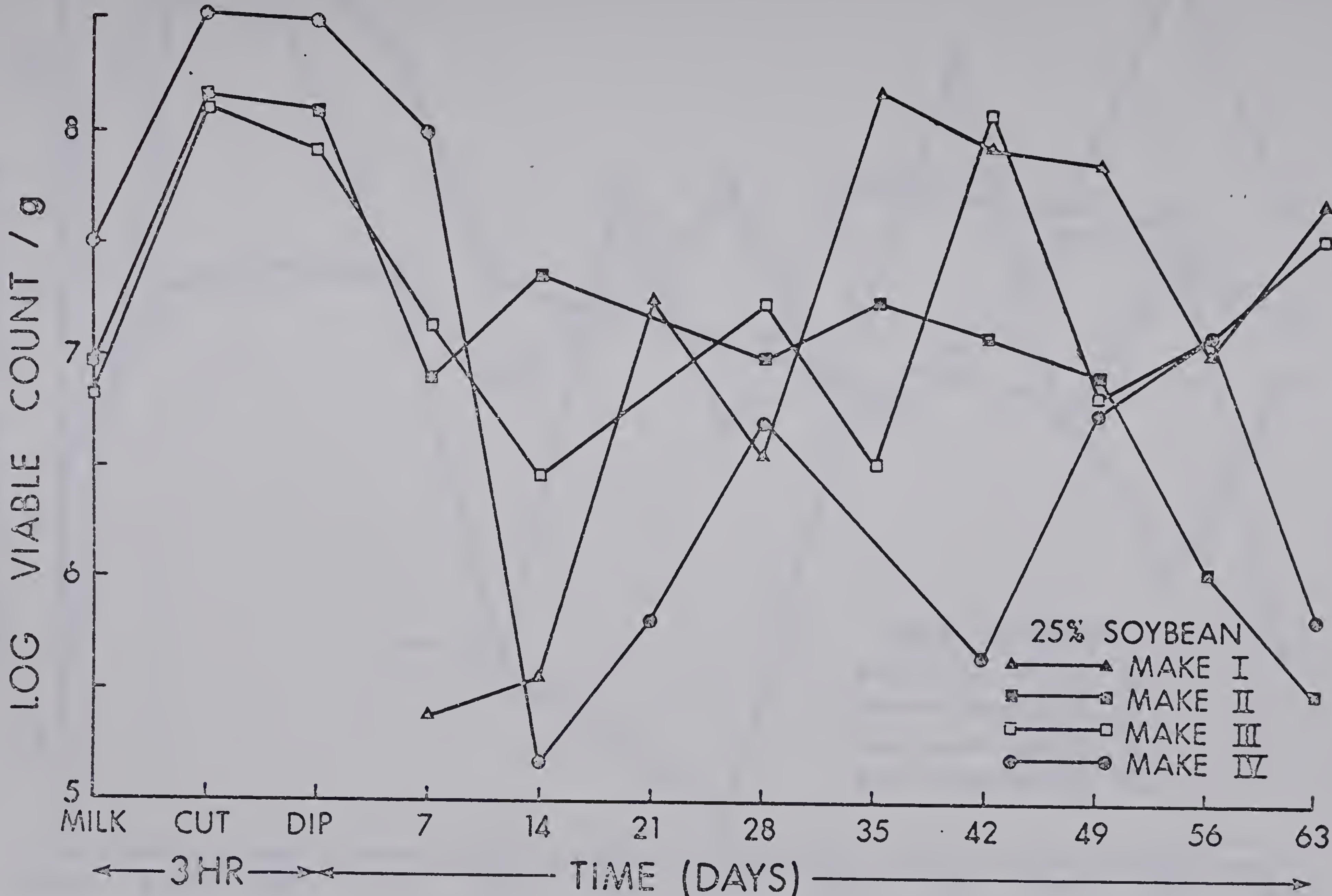


FIGURE 13: CHANGES IN BACTERIAL NUMBERS DURING MANUFACTURE AND RIPENING OF SOYBEAN-SKIMMILK CHEESES.

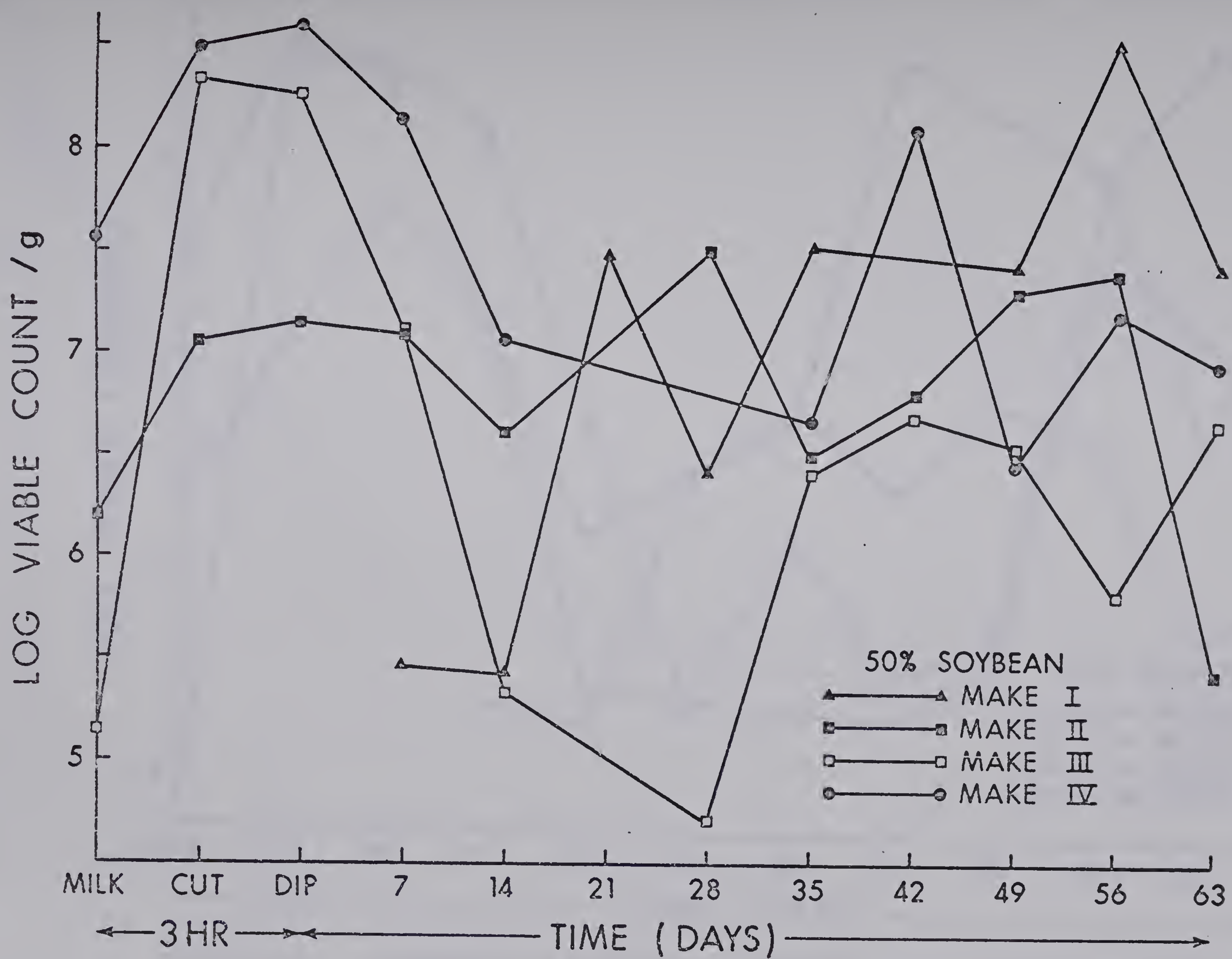


FIGURE 14: CHANGES IN BACTERIAL NUMBERS DURING MANUFACTURE AND RIPENING OF SOYBEAN-SKIMMILK CHEESES.

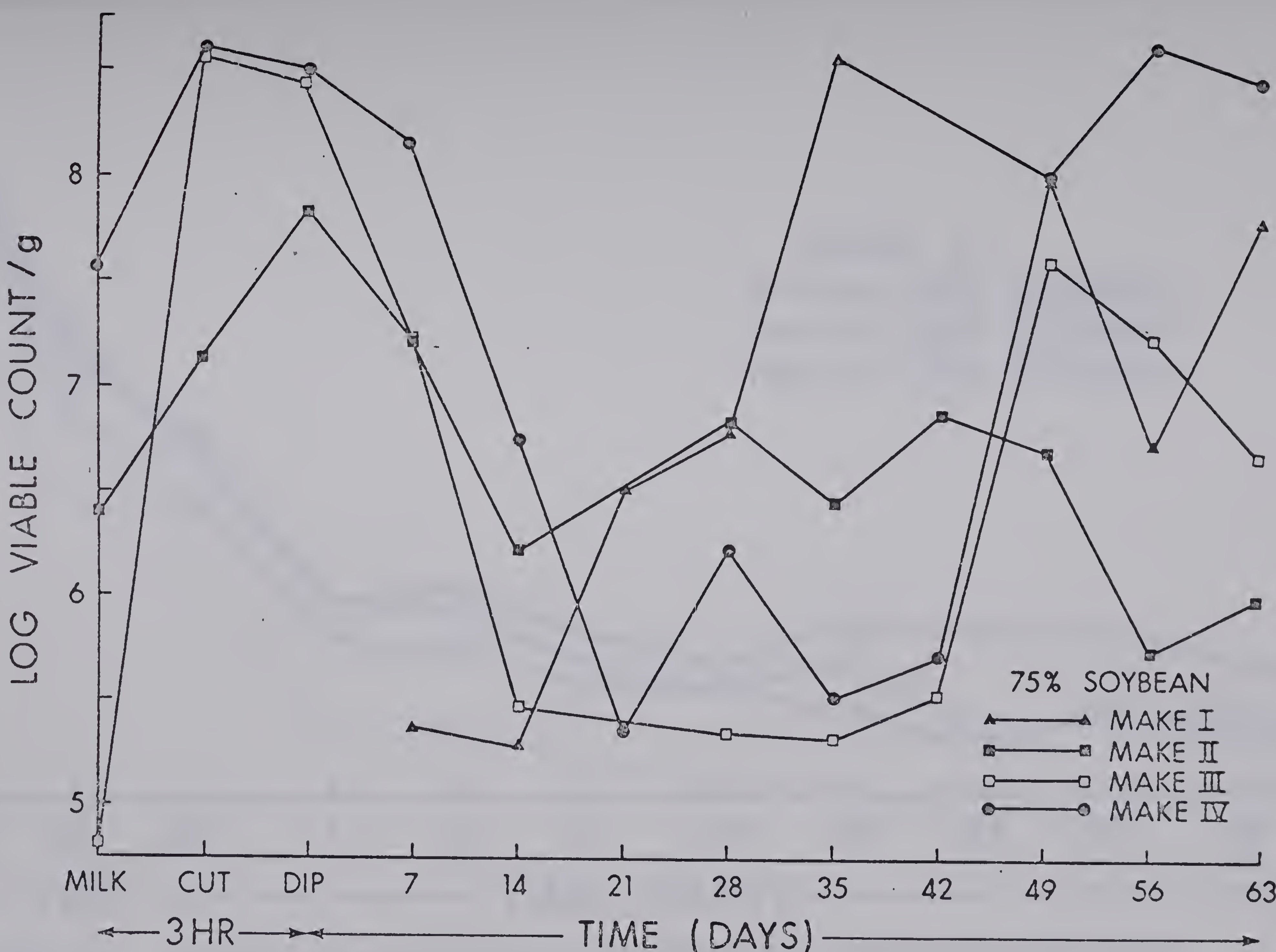


FIGURE 15: CHANGES IN BACTERIAL NUMBERS DURING MANUFACTURE AND RIPENING OF SOYBEAN-SKIMMILK CHEESES.

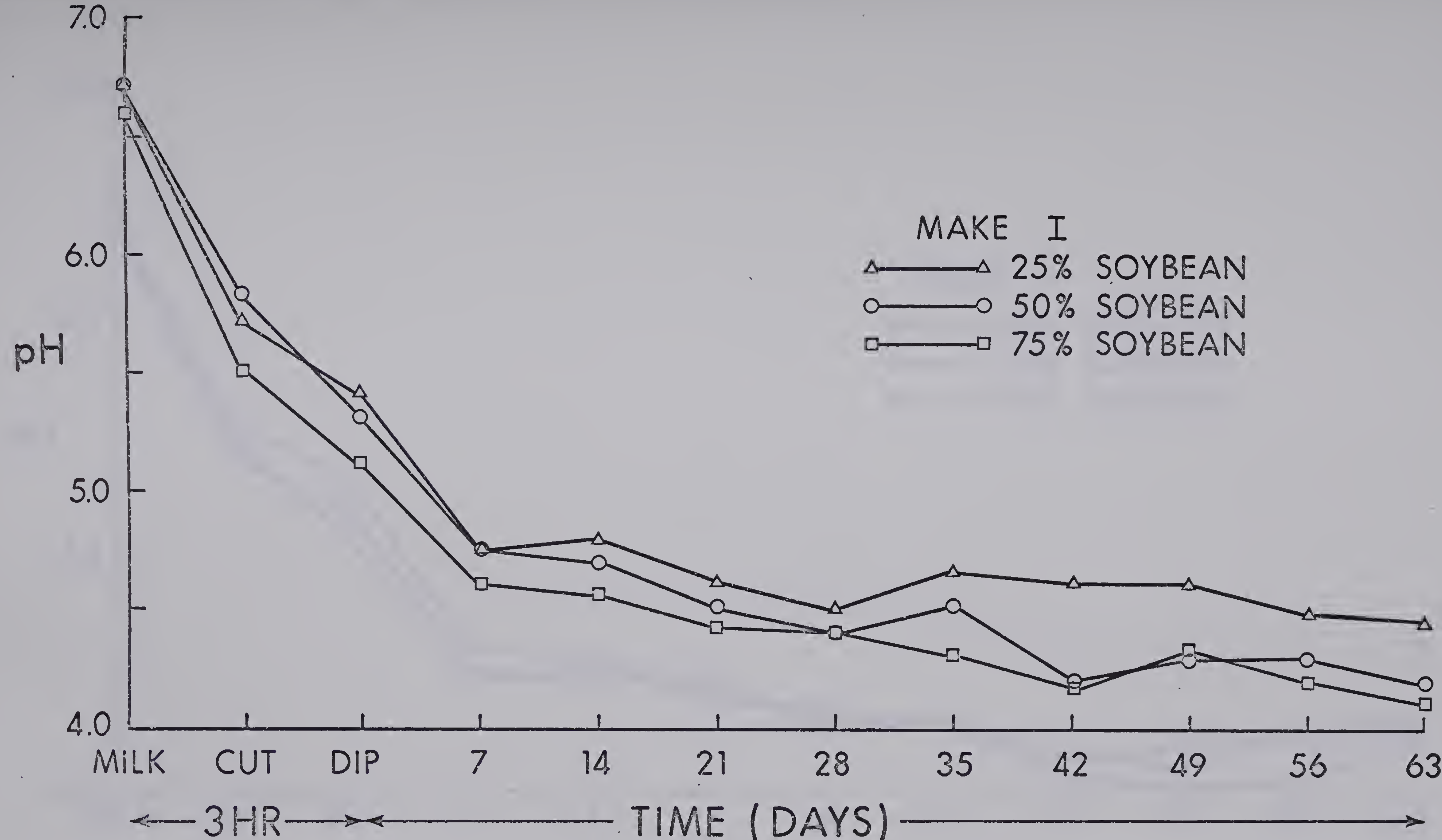


FIGURE 16: CHANGES IN pH DURING MANUFACTURE AND RIPENING OF SOYBEAN-SKIMMILK CHEESES.

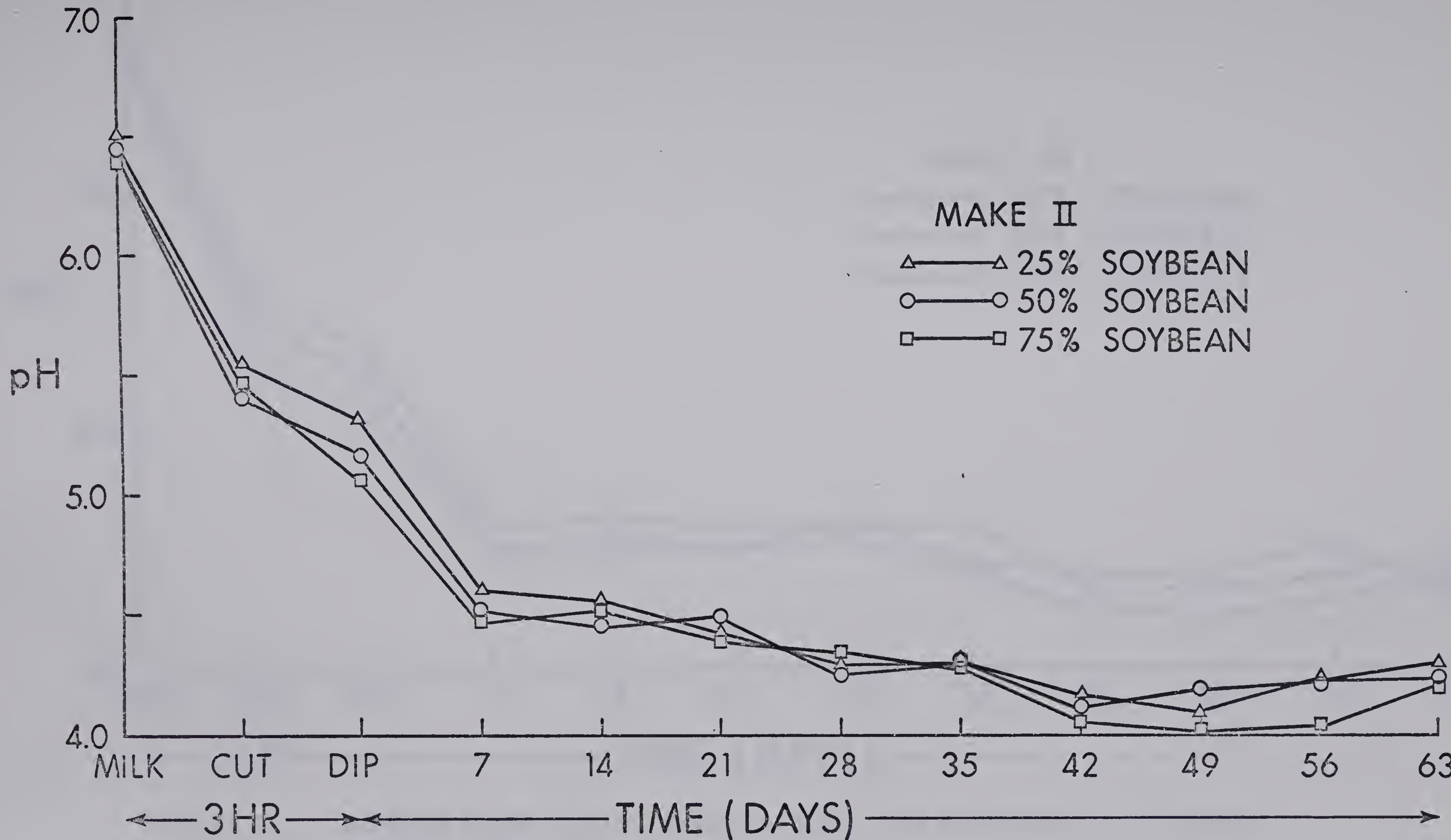


FIGURE 17: CHANGES IN pH DURING MANUFACTURE AND RIPENING OF SOYBEAN-SKIMMILK CHEESES.

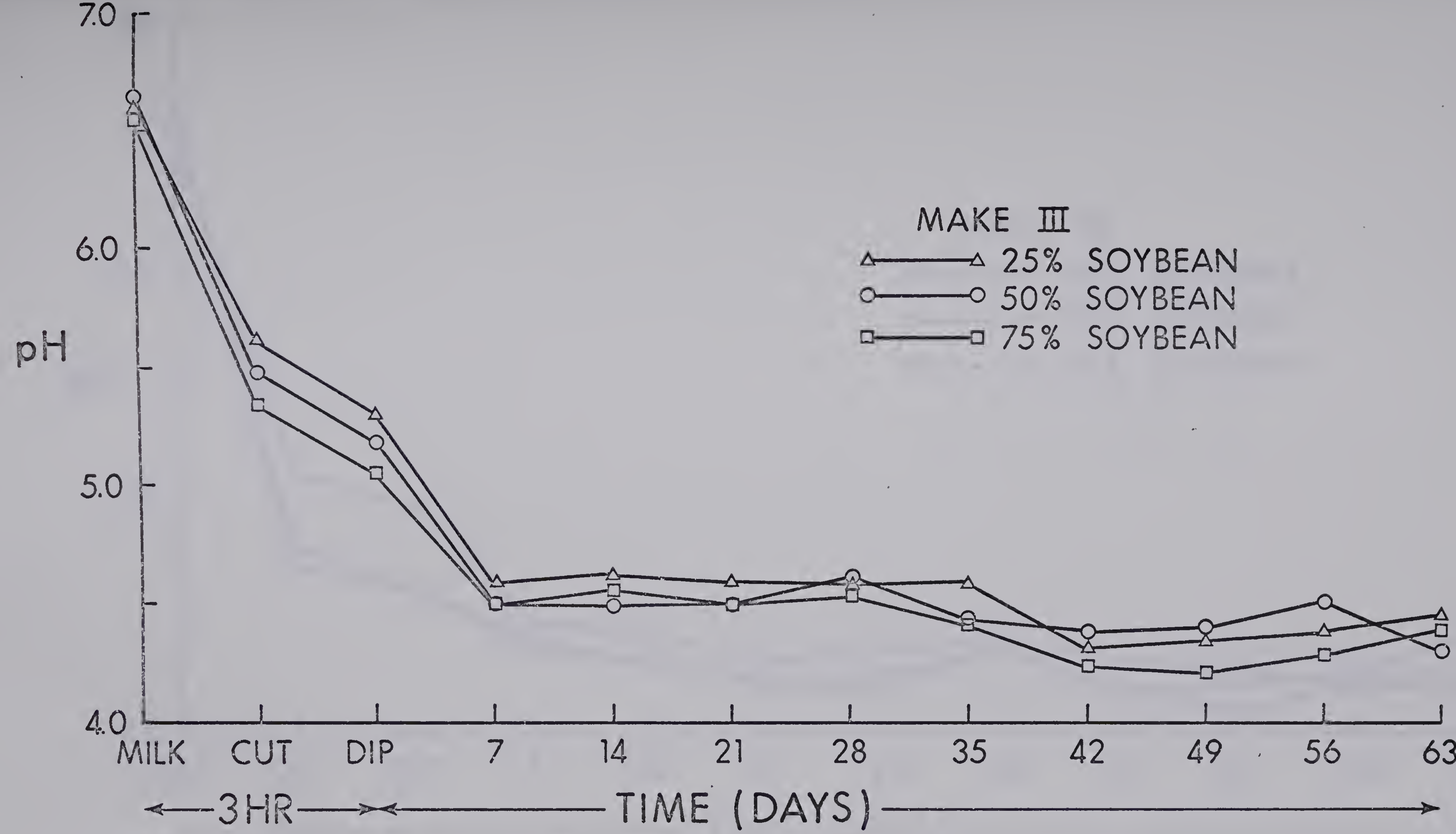


FIGURE 18: CHANGES IN pH DURING MANUFACTURE AND RIPENING OF SOYBEAN-SKIMMILK CHEESES.

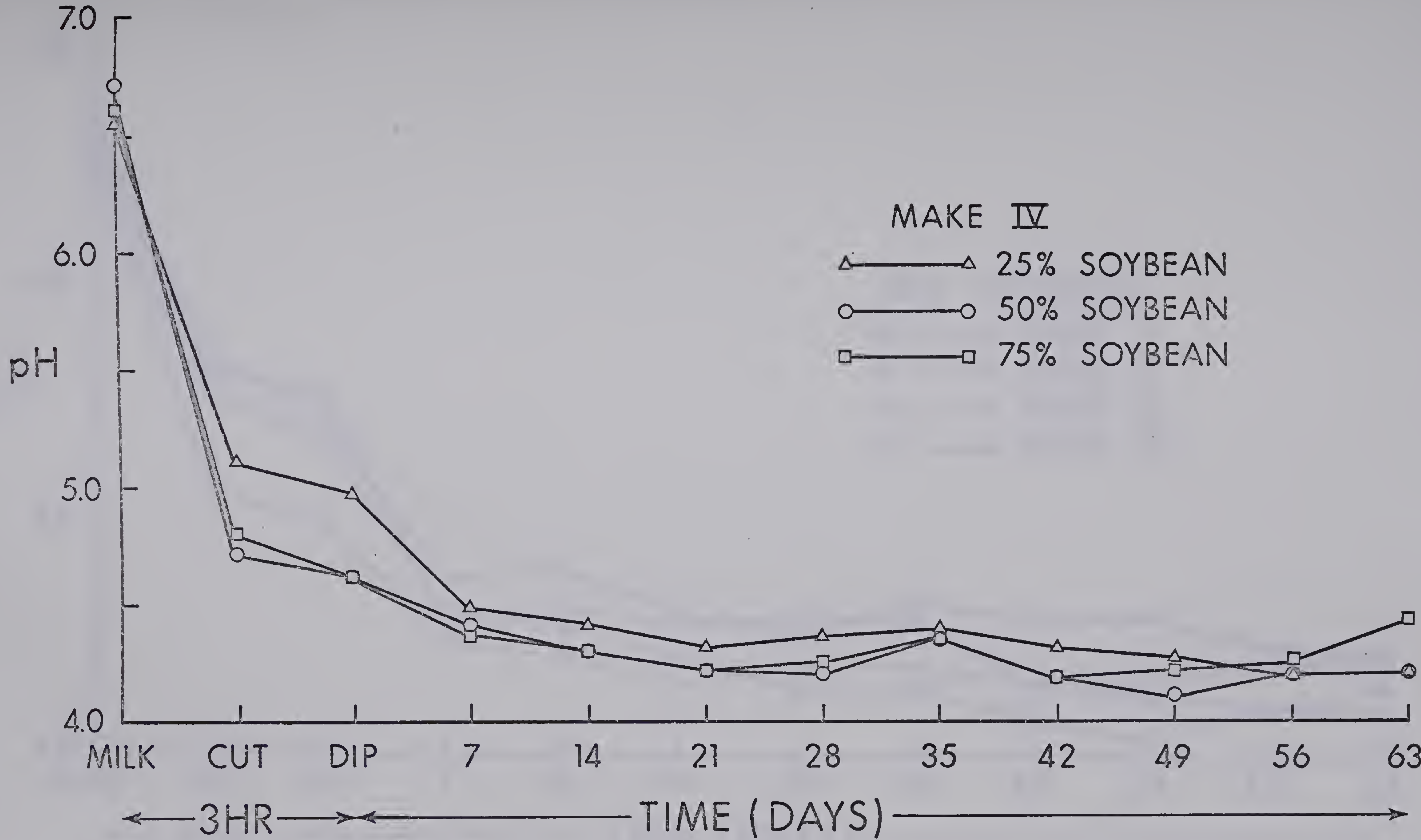


FIGURE 19: CHANGES IN pH DURING MANUFACTURE AND RIPENING OF SOYBEAN-SKIMMILK CHEESES.

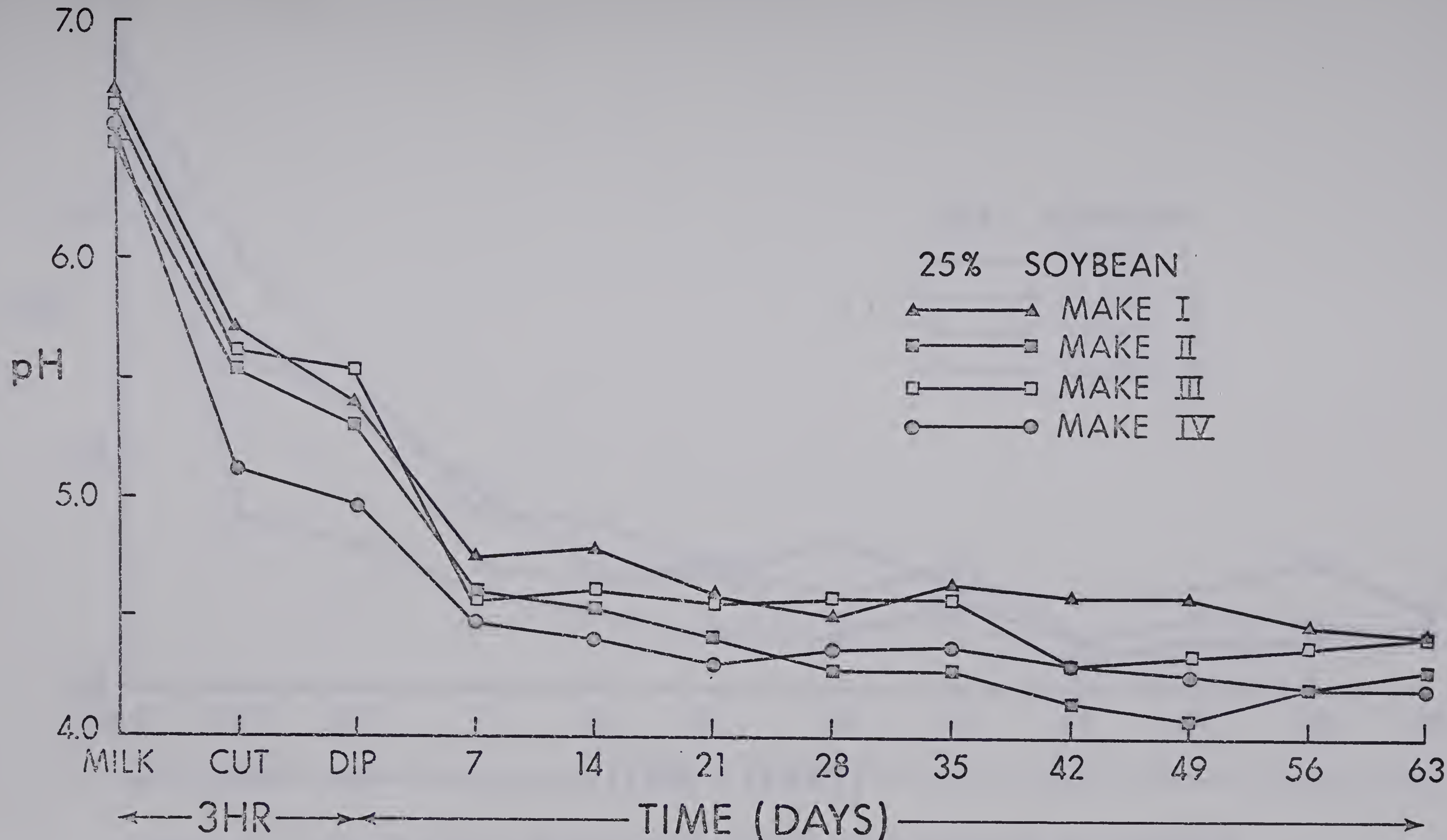


FIGURE 20: CHANGES IN pH DURING MANUFACTURE AND RIPENING OF SOYBEAN-SKIMMILK CHEESES.

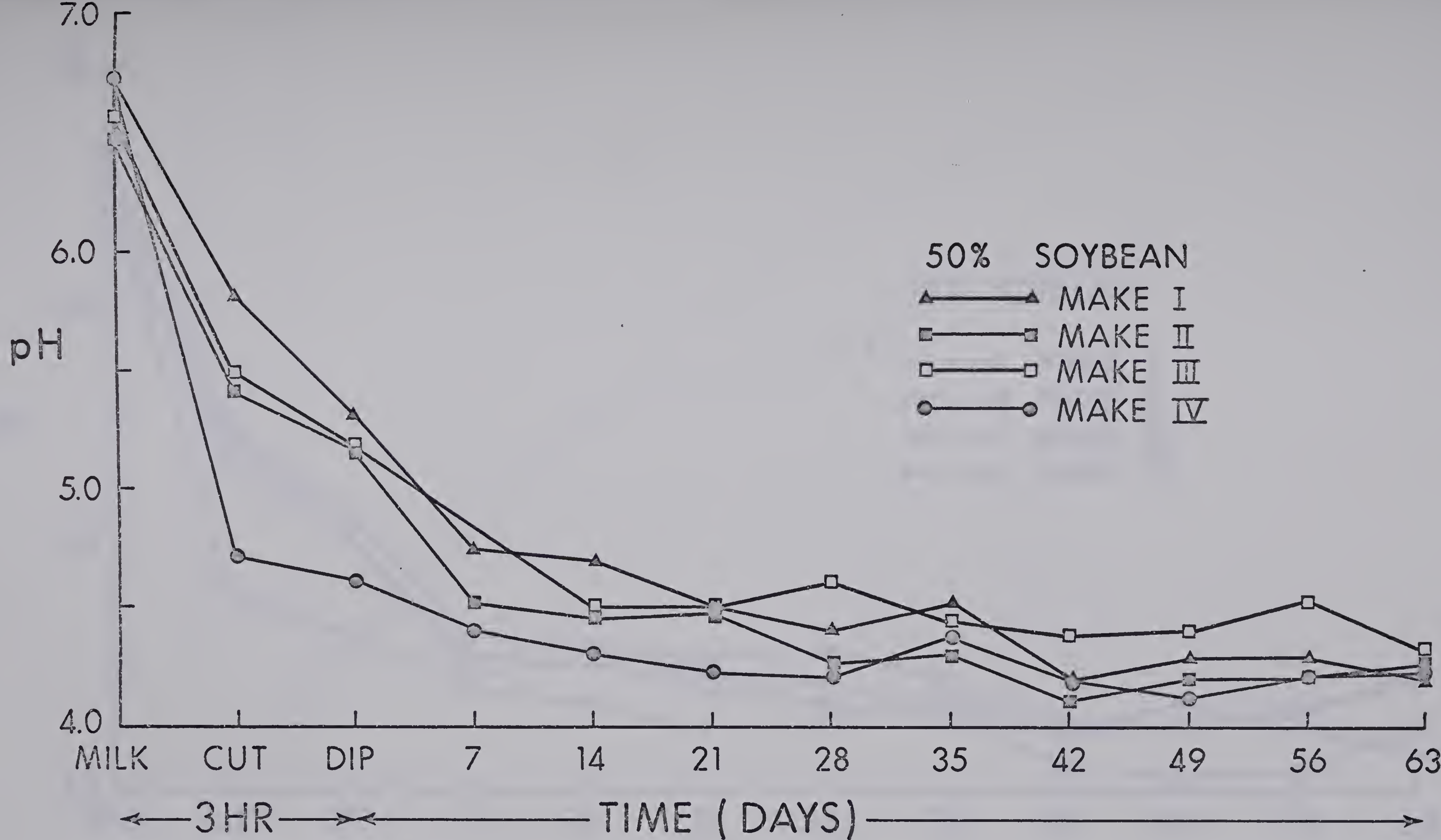


FIGURE 21: CHANGES IN pH DURING MANUFACTURE AND RIPENING OF SOYBEAN-SKIMMILK CHEESES.

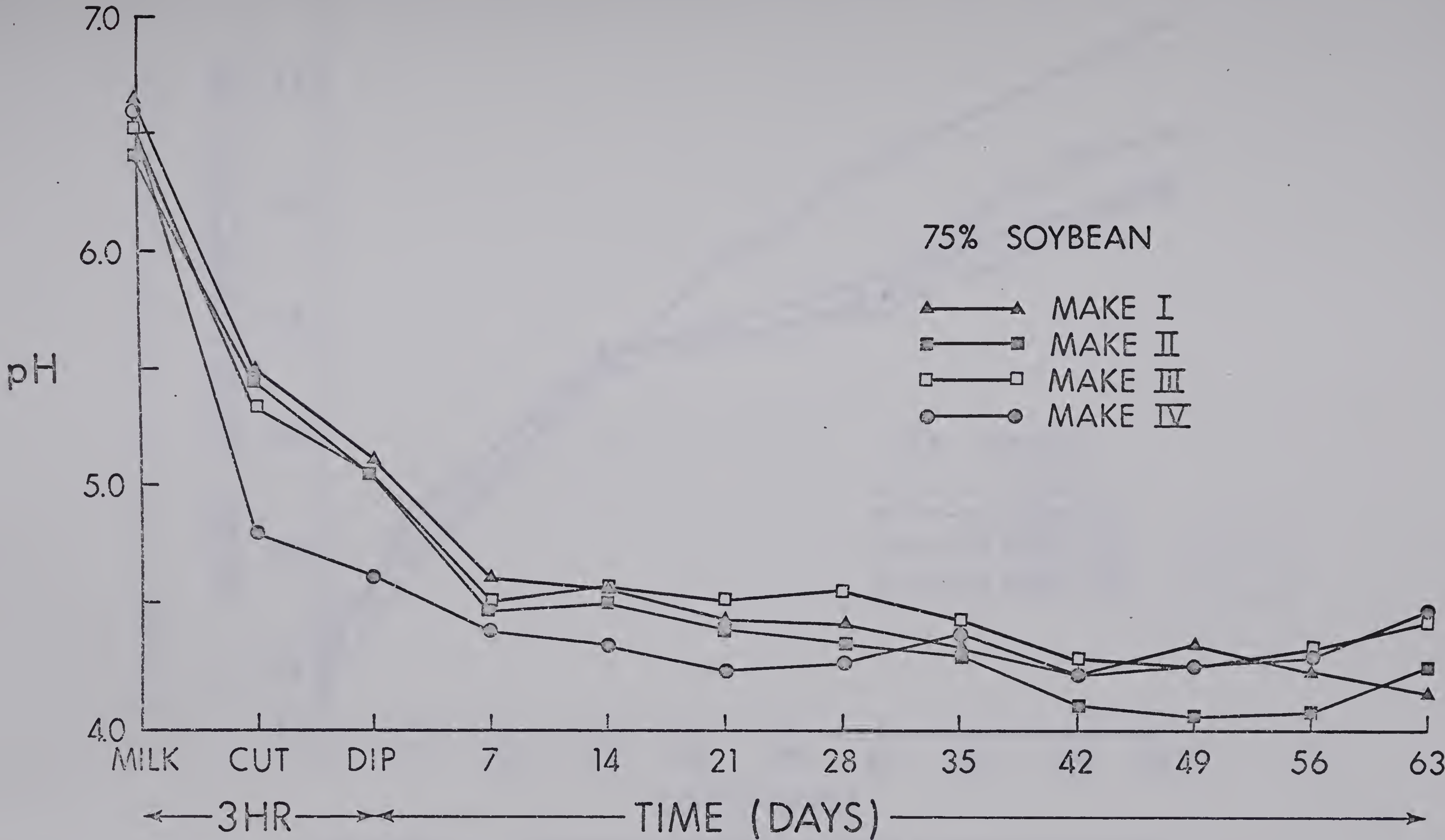


FIGURE 22: CHANGES IN pH DURING MANUFACTURE AND RIPENING OF SOYBEAN-SKIMMILK CHEESES.

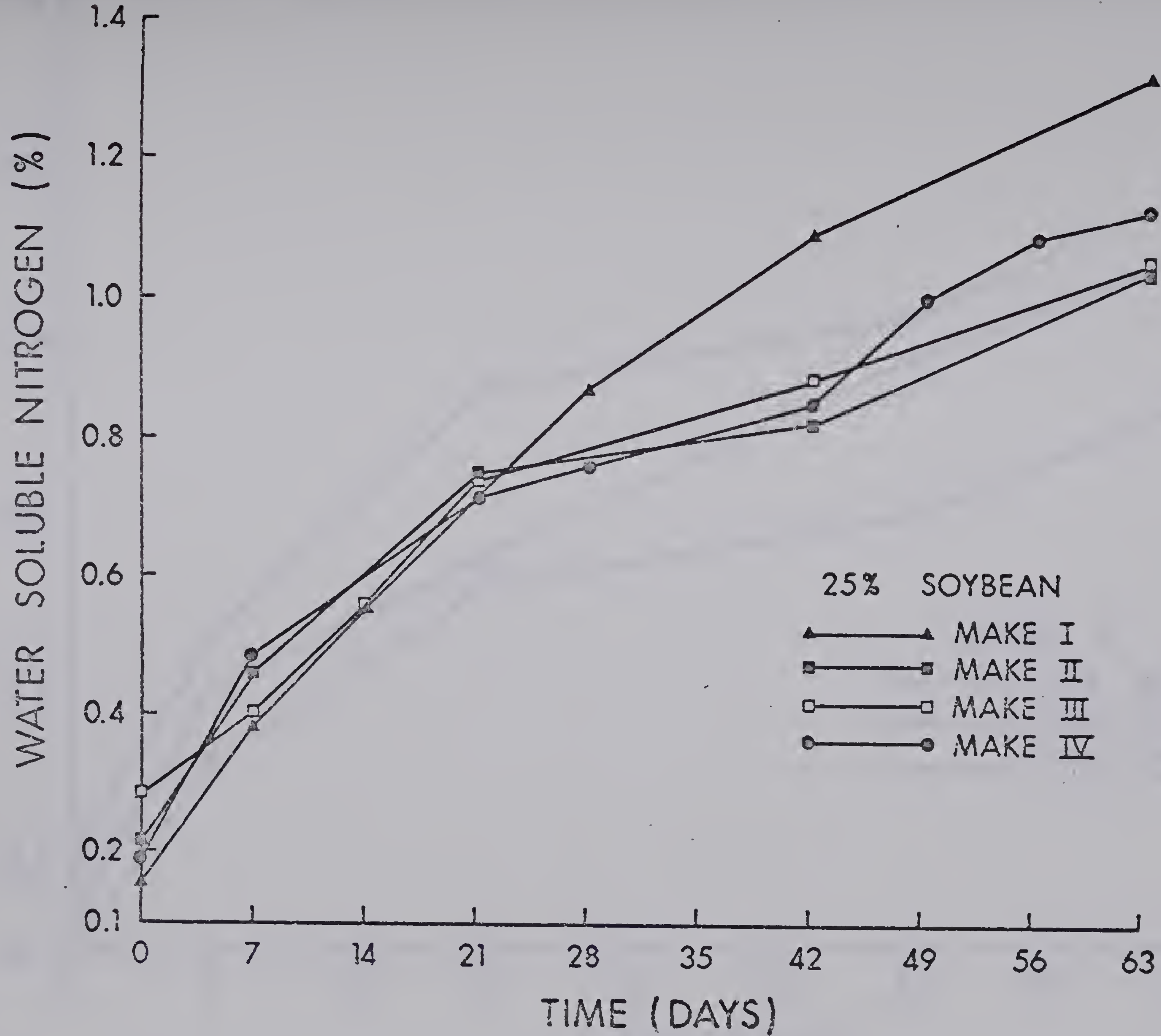


FIGURE 23: CHANGES IN WATER SOLUBLE NITROGEN DURING RIPENING OF SOYBEAN-SKIMMILK CHEESES.

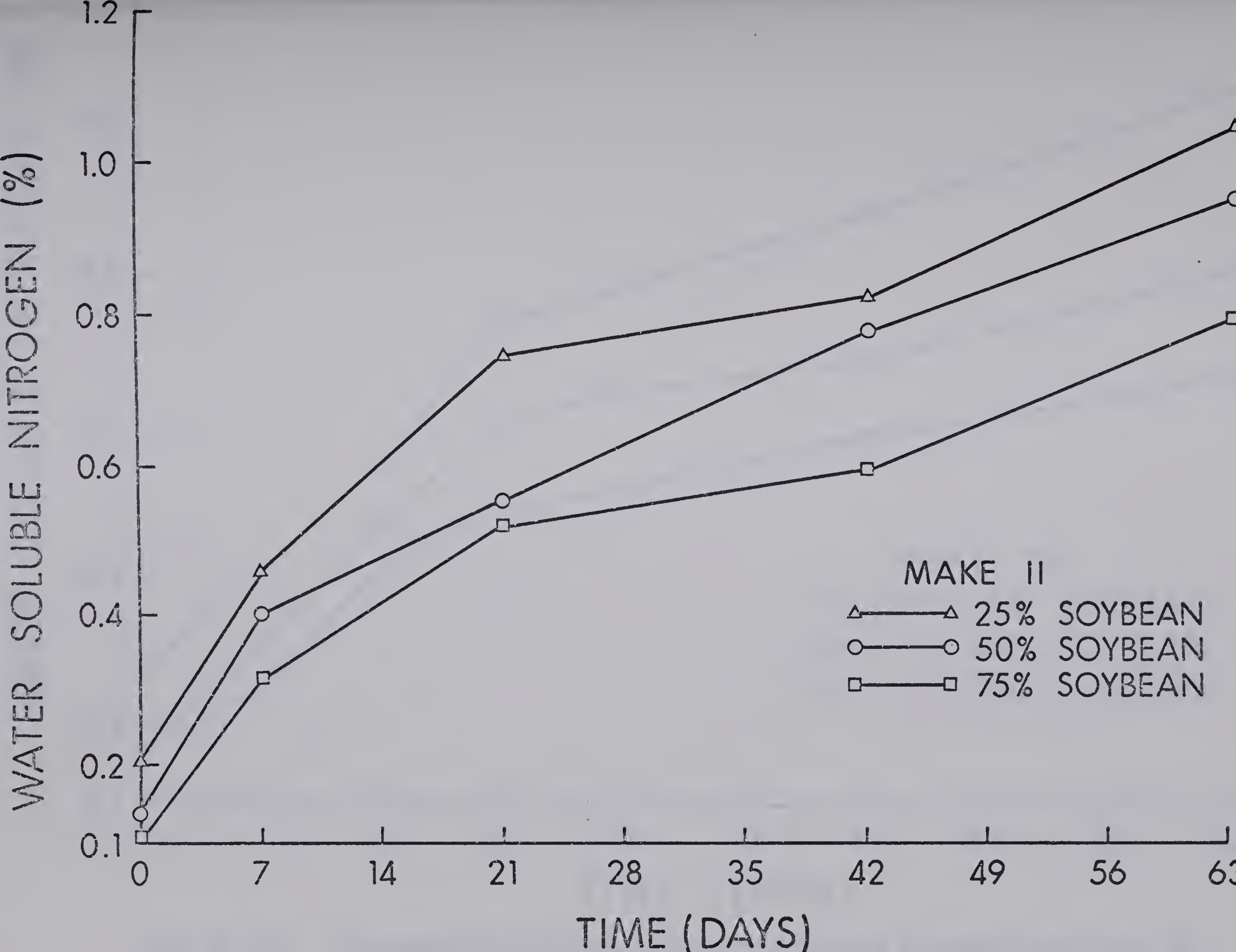


FIGURE 24: CHANGES IN WATER SOLUBLE NITROGEN DURING RIPENING OF SOYBEAN-SKIMMILK CHEESES.

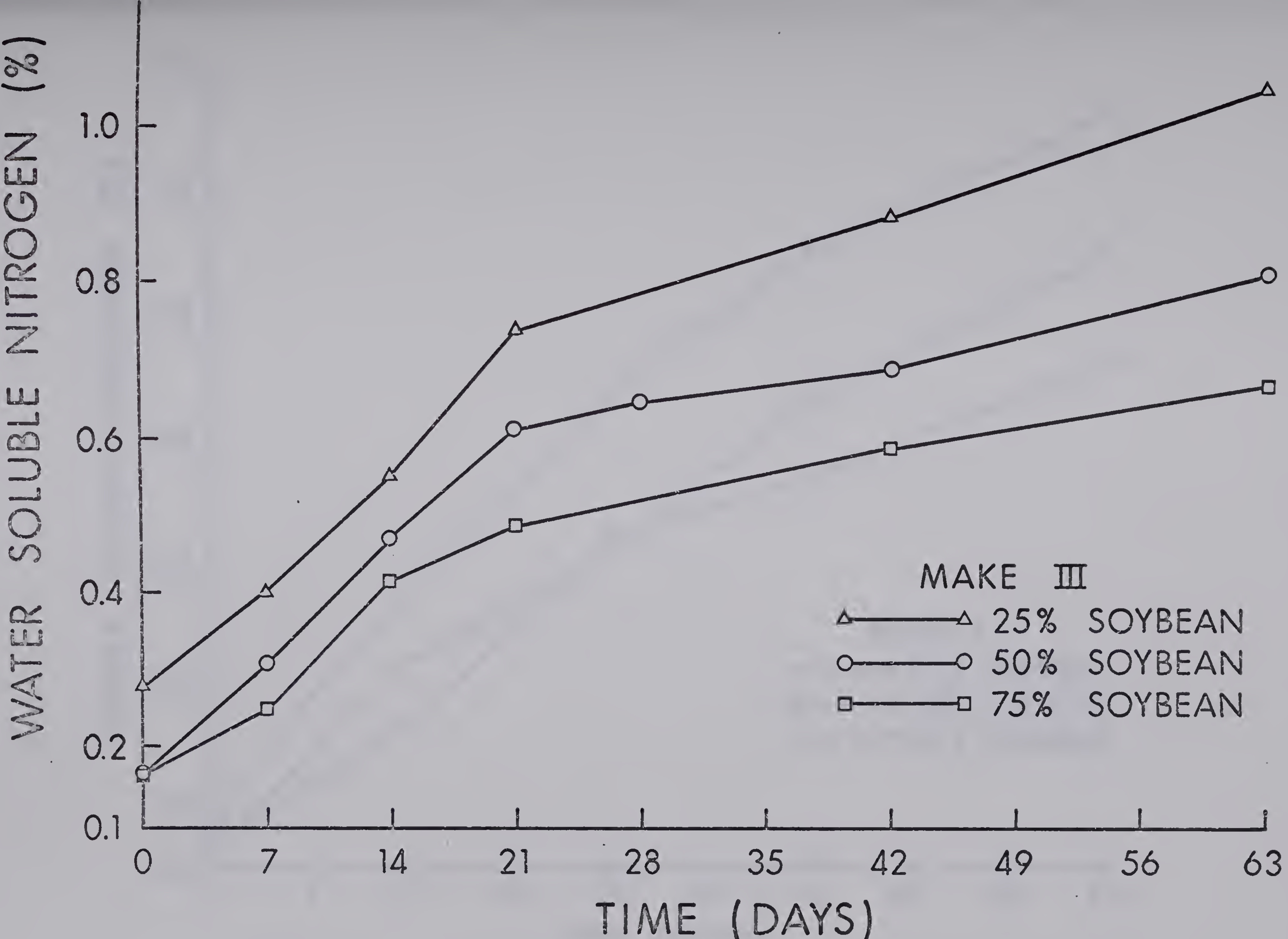


FIGURE 25: CHANGES IN WATER SOLUBLE NITROGEN DURING RIPENING OF SOYBEAN-SKIMMILK CHEESES.

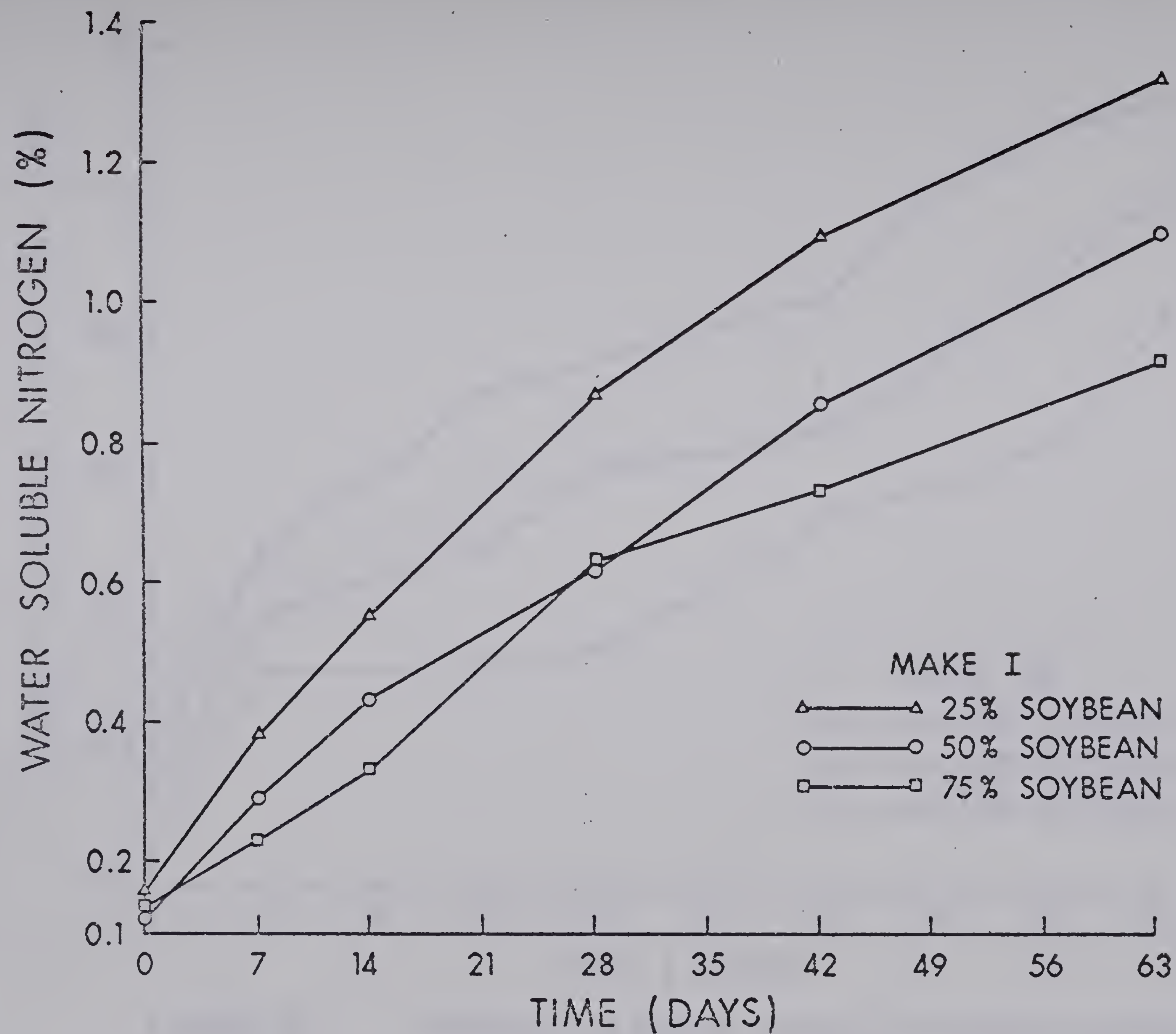


FIGURE 26: CHANGES IN WATER SOLUBLE NITROGEN DURING RIPENING OF SOYBEAN-SKIMMILK CHEESES.

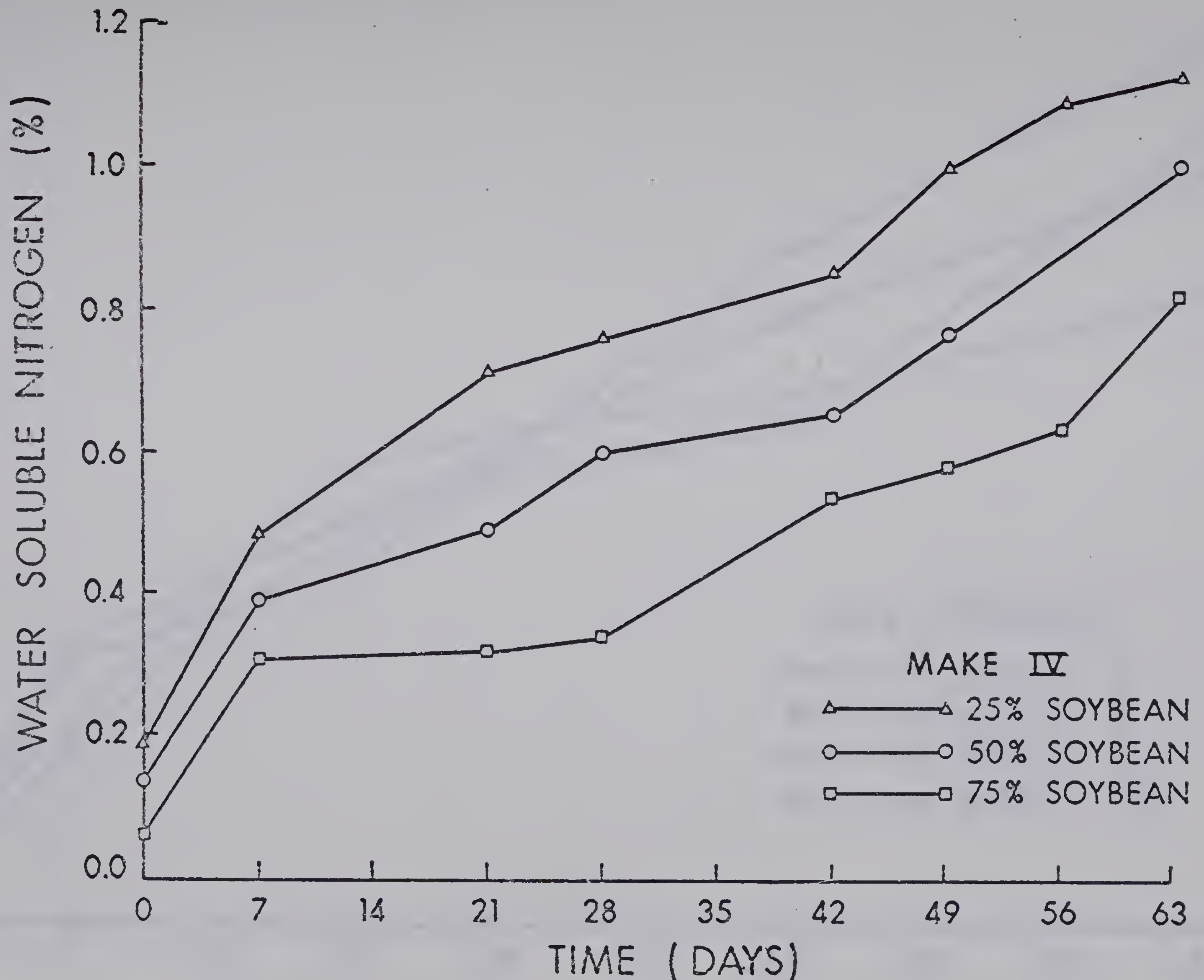


FIGURE 27: CHANGES IN WATER SOLUBLE NITROGEN DURING RIPENING OF SOYBEAN-SKIMMILK CHEESES.

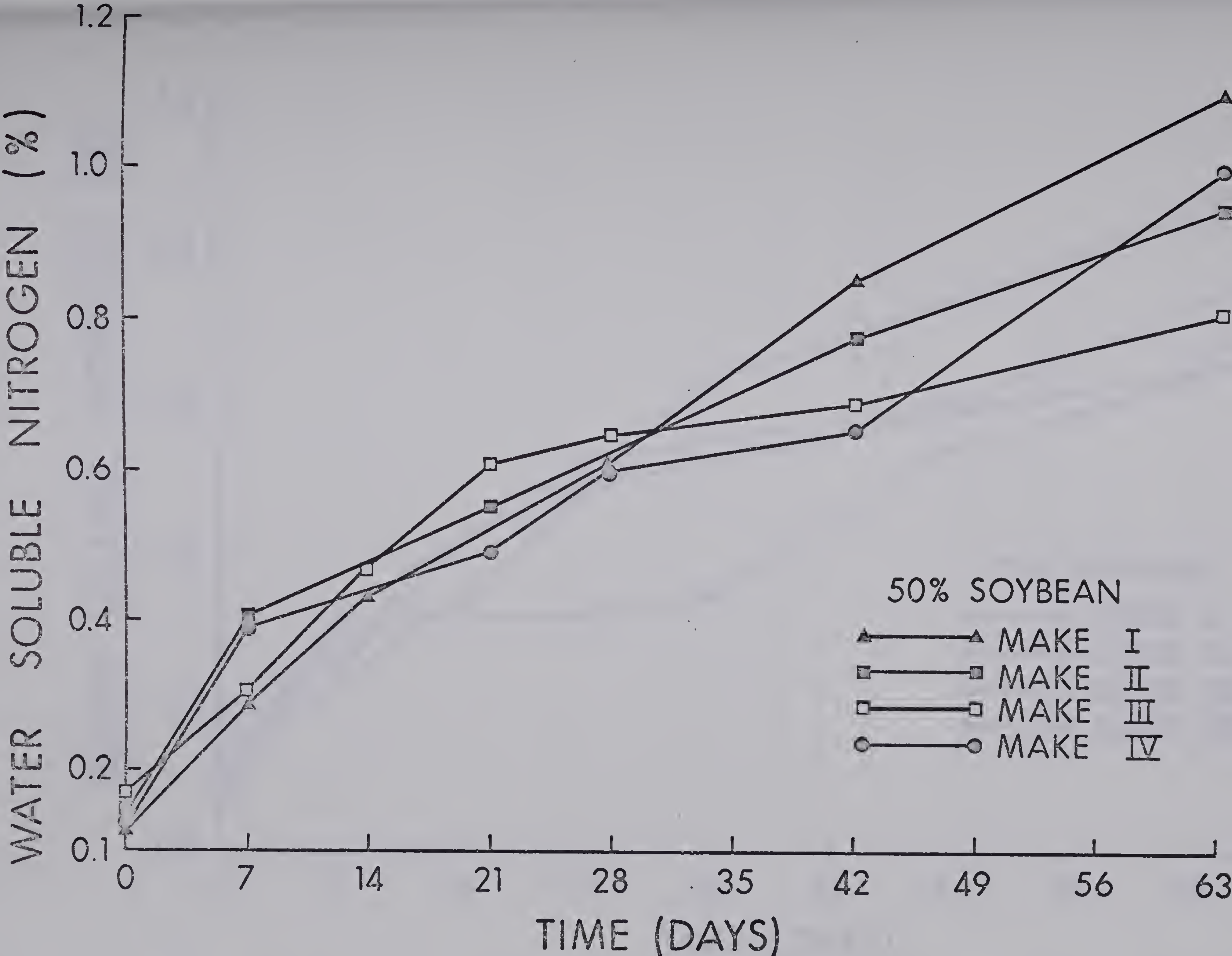


FIGURE 28: CHANGES IN WATER SOLUBLE NITROGEN DURING RIPENING OF SOYBEAN-SKIMMILK CHEESES.

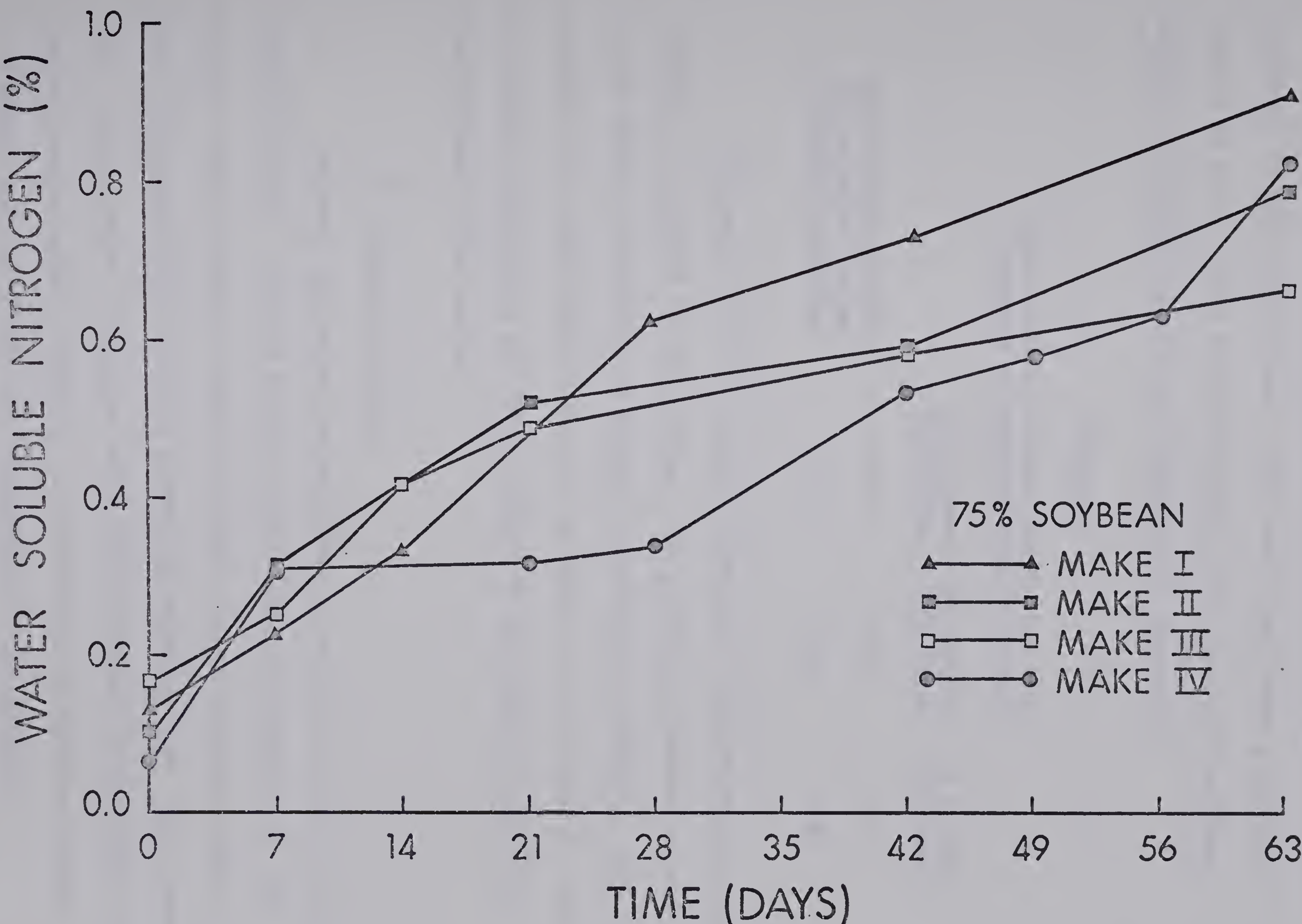


FIGURE 29: CHANGES IN WATER SOLUBLE NITROGEN DURING RIPENING OF SOYBEAN-SKIMMILK CHEESES.

c) Water soluble nitrogen

Water soluble nitrogen determinations were made at weekly intervals on the stored cheese. The results are shown graphically in Figures 23 to 29. Each curve represents the results of duplicate analysis. Detailed results are tabulated in Appendix C.

Table VI

Water Soluble Nitrogen Before and After Ripening of Soybean-Skimmilk Cheeses

	25% Soybean (75% Skim)	50% Soybean (50% Skim)	75% Soybean (25% Skim)
Initial H_2O soluble N	0.21	0.14	0.11
H_2O soluble N after 63 days ripening	1.14	0.97	0.80

The water soluble nitrogen, which is an indication of protein breakdown, increased during storage. The greatest breakdown occurred in the cheeses containing the highest percentage of skimmilk. This is probably due to more selective action of rennet on the skimmilk protein. The higher carbohydrate and fiber content of the soybean cheeses may also influence these values.

d) Moisture content

Moisture determinations were made at weekly intervals on the ripened cheeses. Detailed results are tabulated in Appendix D. The moisture content remained relatively stable during ripening. This is due to the waxed cheese surfaces preventing moisture loss. The cheeses with a higher amount of soybean had a higher moisture content than the cheeses containing more skimmilk. The difference in moisture content is probably associated with the differences in the physical

characteristics of the curd. The high water binding capacity of soybean protein could also be a factor.

e) Flavor and texture

The soybean-skimmilk cheeses were fairly bland in flavor but gradually acquired bitter and beany flavors during ripening. The beany flavor was not diminished by increasing concentrations of skimmilk. This is a surprising result that demonstrates the potency of the beany flavor of soybeans. The development of bitter flavors during ripening was probably a result of protein hydrolysis yielding bitter peptides. This is borne out in Table VI showing the increase in water soluble nitrogen during ripening.

The texture was slightly floury, becoming more pronounced with increasing amounts of soybean in the cheese. This is probably due to the carbohydrate and fiber fraction of the soybean.

iii) Mold Ripening

Excellent surface growth was obtained in all the surface ripening experiments. The surface ripened samples soon became very bitter in flavor. The texture, however, was greatly improved. The floury texture was reduced due to a breakdown of the carbohydrate and fiber fraction by the mold. The tempeh extract cheese tasted slightly sweet at the extract stage but the cheese developed bitter flavors on storage. The texture was similar to the surface ripened samples. The water soluble nitrogen after three weeks of storage in all the mold ripened cheeses was much higher than the control. Table VII shows the average of two cheeses in each test.

Table VII

Water Soluble Nitrogen of Mold Ripened Soybean Cheeses

	% Water Soluble N	
	Initial	3 Weeks Ripening
Penicillium camemberti surface ripening	0.10	1.43
Rhizopus oligosporus surface ripening	0.10	0.81
Tempeh extract cheese	0.80	1.09
Control	0.10	0.28

The undesirable flavors produced were probably due to the mold enzyme hydrolysis of the protein giving the formation of bitter peptides, etc.

Soybean Curd Prepared by Using Calcium Sulfate

i) Characteristics of the Soybean Curd Produced

The curd produced had a very slight beany flavor and could be termed bland. The texture was varied by the amount of pressure applied to the hoops. A pressure of over 1 psi caused the curd to be rubbery and tough.

The analysis of the soybean curd is shown in Tables VIII and IX.

Table VIII

Analysis of Soybean Curd*

Protein (D.W. basis)(N x 6.25)	55.6 %
Fat (D.W. basis)	31.9 %
Moisture	76.8 %
H_2O soluble N	0.06%
pH average	6.01
% yield	31.7 %
Penetrometer	126

* Average of six samples.

Table IX

Amino Acid Analysis of Soybean Curd

Amino Acid	% of Total Curd Protein	Amino Acid	% of Total Curd Protein
Lysine	6.18	Alanine	4.22
Histidine	2.37	Half cystine	2.26
Arginine	7.25	Valine	5.31
Aspartic acid	11.62	Methionine	0.99
Threonine	3.60	Isoleucine	4.95
Serine	4.65	Leucine	8.02
Glutamic acid	19.53	Tyrosine	3.60
Proline	3.27	Phenyl alanine	5.33
Glycine	3.92		

The protein content using the calcium sulfate precipitation method produced a uniform product. The analyses of protein of eight separate "makes" gave a coefficient of variation of 2%. (Appendix F)

The amino acid analysis compares closely to an analysis of similar method for producing a soybean curd (Hackler et al., 1967). The methionine content is, however, lower than expected. This will be interpretated later in the nutritional studies.

The low yield in Table VIII is due to the large amount of protein and insoluble material removed during straining. This is illustrated in an analysis of the residue in Table X. The yield may be increased by more than one extraction of the residue.

Table X

Analysis of the Residue from the $CaSO_4$ Precipitation Method

	%
Protein (air dry basis)	34.4
Crude fiber	28.1
Crude fat	13.6

The effects of storing the waxed curd samples at 20 C are shown in Table XI.

Table XI

Analysis of Soybean Curd After Six Weeks Storage at 20°C *

Moisture	74.44 %
pH	5.54 %
H_2O soluble N	0.25 %
Penetrometer	148

* Average of 6 samples.

There was no difference in the keeping quality or analysis between the salted and unsalted curds. The stored curd samples had a slight off-flavor. There was a slight increase in water soluble nitrogen and a decrease in the pH denoting some spoilage.

ii) Taste Panel Evaluation

The comparative beaniness of the test curd and Chinese soybean curd was evaluated by 23 panel members. Eighteen termed the test curd less beany than the Chinese curd. Four found it more beany and one found no difference between the samples. To avoid assigning absolute numbers to the extent of the beany flavor, the data was treated as non-parametric. The statistical methods used to analyze the data were the sign test and Wilcoxon's sign rank test. In both sign and Wilcoxon's sign rank tests, beany flavor was highly significantly less in the test curd than in the Chinese curd sample (to the 1% level). The procedure is shown in Appendix G.

The results of the texture study show that the test curd was described as follows: firm (14), rubbery (7) and smooth (7). The majority marked the Chinese soybean curd spongy (11), smooth (5),

rubbery (4) and mealy (4).

11 members preferred Sample I - Test
8 members preferred Sample III - Test
4 members preferred Sample II - Chinese Soybean Curd

Eighteen members were able to distinguish which sample was different. It may be noted that all members of the taste panel of Oriental extraction preferred the Chinese soybean curd over the test samples. This was a difficult product to assess as it was not a finished product nor was it one which was familiar to the panel members. The basic difference between soybean curd and Chinese soybean curd is its lack of beany flavors and its firmer texture.

iii) Nutritional Studies

The data for the individual rats was averaged and presented in Table XII. The detailed results are tabulated in Appendix E.

Table XII

Feed Intake, Gains, Feed Conversion and Protein Efficiency Ratio of Rats Fed Diets Containing Casein or Soybean Curd

	Control			Test		
	M	F	M & F	M	F	M & F
Average daily feed g	13.75	11.38	12.57	10.76	10.70	10.73
Average daily gain g	6.88	4.84	5.87	4.37	3.88	4.13
Feed conversion g	2.00	2.35	2.18	2.46	2.76	2.60
Total feed wastage g	13.00	30.00	21.50	322.00	97.00	209.50
Protein efficiency ratio $\left(\frac{\text{grams gained}}{\text{grams protein consumed}}\right)$	2.19	1.86	2.02	1.80	1.62	1.71

In both diets, the male rats consumed more feed, gained faster and were more efficient in feed conversion than were the females. A summary of the degree of significance of the results obtained is shown in Table XIII. The procedure for the analysis of variance is shown in Appendix H.

Table XIII

Nutritional Study - Analysis of Variance

	Source of Variation	F Value
Average daily consumption	sex	10.23 ***
	diet	23.38 ***
	sex x diet	9.27 ***
Average daily gain	sex	39.00 ***
	diet	73.40 ***
	sex x diet	14.63 ***
Consumption / gain	sex	21.02 ***
	diet	53.26 ***
	sex x diet	0.75
Protein efficiency ratio (PER)	sex	65.00 ***
	diet	99.00 ***
	sex x diet	6.00 *
Feed wastage	sex	2.68
	diet	8.75 **
	sex x diet	3.62

* significance to 5% level
** significance to 1% level
*** significance to 0.5% level

All differences attributable to sex were significant ($P \leq .05$) except feed wastage. A significant ($P \leq .05$) sex x diet interaction noted for the average daily consumption, average daily gain and protein efficiency ratio shows there is an interaction between the diet and the sex of the rat. Recognizing the mentioned differences, the rats fed the diet containing casein as the sole source of protein consumed more feed, had higher gains, were more efficient both in terms of feed conversion and protein efficiency ratios than were the rats fed the test diet containing soybean curd as the sole source of protein. All differences were significant ($P \leq .05$). The feed wastage of soybean diet may reflect a palitability factor in the test diet. The poorer performance of the rats fed the diet containing the soybean curd may be

attributable to an unfavorable balance of certain essential amino acids shown in Table IX. Using the figures from the amino acid analysis of the soybean curd and the amino acid composition of casein taken from Crampton and Harris (1969), the amino acid composition of the diets was calculated and compared with the amino acid requirements of the growing rat as set down by the National Academy of Sciences - National Research Council (United States)(1962). This is shown in Table XIV.

Table XIV

Amino Acid Composition (% of Diet)

Amino Acid	Control (Casein) %	Test (Soybean Curd) %	Minimum Requirement (NRC) %
L-tryptophan			0.15
L-histidine	0.72	0.53	0.30
L-lysine	2.03	1.38	0.90
L-leucine	2.50	1.79	0.80
L-isoleucine	1.66	1.10	0.50
L-phenylalanine	1.34	1.19	0.90
L-methionine	0.78	0.22	0.60
L-threonine	1.10	0.80	0.50
L-valine	1.98	1.18	0.70

The test soybean curd diet met the amino acid requirements of the rat for all the amino acids except methionine. The diet only met approximately one third of the rats' requirement for methionine, an essential amino acid for the rat. Deficiency of an essential amino acid will result in reduced feed intake and therefore, reduced growth. This further results in an increased feed conversion ratio and a decreased protein efficiency ratio resulting from less efficient utilization of the dietary protein.

iv) Synthetic Meat Products

An attempt was made to utilize the soybean curd produced by calcium sulfate precipitation for the preparation of synthetic meat products. Various meat spices, together with recommendations on their usage, were kindly donated by Griffith Laboratories Limited, Scarborough, Ontatio, Fritzsche Brothers of Canada, Toronto, Ontario and Food Products Limited, Montreal, Quebec. The flavored curd was stuffed into sausage casings and autoclaved in order to produce a sausage type meat. Problems were encountered during the steaming of the sausage due to bursting of the casings. This was due to the high water binding capacity of the soybean protein. Loose stuffing of the casings and a higher initial moisture content effectively overcame this problem.

Flavored curd was also canned and sterilized. The compression in the can gave a very firm texture to the product.

Both the sausage type "meat" and canned "meat" were evaluated. The sausage "meat" was evaluated informally and was considered to be quite acceptable. The canned "meat" was evaluated by a taste panel consisting of 30 members. The members of the panel were allowed to taste the product as prepared or with bread and butter. The results of the panel were as follows:

Very acceptable	15
Moderately acceptable	13
Neither like nor dislike	2
Total	30

These results demonstrated that, even with limited experience in the science and art of food flavoring, it is possible to produce quite acceptable "meats" from the soybean curd developed in this work.

DISCUSSION AND CONCLUSIONS

Previous work by Hang and Jackson (1967a and b) showed that the addition of skimmilk (15% by weight) and rennet could bring about improvements in flavor and texture of soybean cheese. The skimmilk considerably reduced the time required for the manufacture of the curd. It is also a reasonable assumption that the skimmilk improved the nutritional value of the product. In this work considerably more skimmilk was incorporated into the cheese (25 - 75% by weight) with a view to bringing about further improvements in the characteristics of the finished product.

The amount of skimmilk had little effect on the flavor of the cheese due to the dominating beany flavor of the soybeans. Similarly, the skimmilk had little effect on the texture of the cheese. This would indicate that only a small amount of the fibrous material associated with soybeans is necessary to impart a mealy texture to the product. The skimmilk did, however, considerably reduce the manufacturing period due to the more rapid coagulation of the curd. The effect of the skimmilk in this respect is probably threefold, firstly, the larger the amount of skimmilk would provide a more suitable medium for the starter culture; secondly, rennet has a degree of specificity for casein and hence, its activity would be more apparent in the presence of increasing concentrations of skimmilk; thirdly, the isoelectric point of casein is higher than that of the major proteins of soybean and hence, less time is required to reach the optimum pH for precipitation.

The bitter flavors in the ripened cheeses were probably due

to the formation of peptides, resulting from protein degradation. This corresponds with the increase in the water soluble nitrogen. The breakdown is probably due to the action of the rennet extract. This process would be accelerated by the low pH resulting from the production of lactic acid by the starter bacteria. According to Sumner (1951), the optimum pH for the proteolytic activity of rennin is 3.7. Rennin may also digest some plant globulins. Pepsin, which is present in lesser amounts in rennet, acts on all native protein although the optimum pH is quite low (pH 1.5-2). The starter organisms themselves are non-proteolytic.

The advantages in the fermentation of soybeans for the production of an edible food material have been discussed by Murata et al. (1967) and Hesseltine and Wang (1967). Fermentation may result in the reduction or masking of the beany flavor and the enhancement of the nutritional value of the product. However, the new flavors resulting from fermentation may be acceptable only to some palates. Work by Fujimaki et al. (1968), employing three well known enzymes, papain, bromelin and pepsin, and nine proteolytic enzyme preparations of microbial origins, showed that the beany flavor could be reduced by enzymatic action as well as by fermentation. The major difficulty in either fermentation or enzymatic modification is the control of proteolysis to prevent the formation of bitter peptides. This is confirmed by the results of the experiments on the mold ripening of soybean cheeses. Significant improvements were apparent in the texture of the product but the flavor soon became very bitter and unacceptable. The problems associated with the flavor and texture of the soybean-skimmilk cheeses suggested another approach to the utilization of

of soybeans for human consumption.

In order to develop a more acceptable product, it was considered necessary to eliminate the factors responsible for the undesirable characteristics of the finished cheese, namely, the beany flavor, the mealy texture and the formation of bitter flavor substances resulting from proteolysis. To improve the flavor it was necessary to have a bland soybean milk for the preparation of the cheeses. This was accomplished by elimination of the preliminary steaming of the beans and subsequent dehulling that was used in the original process. It was considered that the original treatment activated the lipoxidase enzymes and thereby, resulted in the production of beany flavors. The blending of the whole beans in water at 80-100°C presumably resulted in the rapid inactivation of the lipoxidase enzyme.

With the elimination of the dehulling process, it was necessary to remove the coarse particulate matter present in the soybean milk. The removal of the particulate matter was reflected in the improved texture of the finished product. The residue has a very high protein value (Table X). This is in agreement with the work of Hackler et al. (1963) who showed that the nutritional value of the residue was higher than the precipitated curd. The residue can have many uses, i.e. animal feed, further refining to remove oil and protein, etc.

Evaluation of the curd resulting from calcium sulfate precipitation of soybean milk prepared with the modifications described above clearly showed that the beany flavor was removed and that the product had a smooth texture.

The formation of bitter flavor compounds as a result of enzymatic or microbial degradation of proteins were eliminated by heat

treatment of both the soybean milk and the finished product. The final product, packaged in a suitable container to prevent further contamination, would hence have a considerable shelf life.

The nutritional study showed the soybean curd to be inferior to casein with respect to nutritional value. As the heat treatment was well above that required for the destruction of growth inhibitors, (Klose et al., 1948) it is assumed that they are not present in the curd. The low methionine content would, therefore, appear to be the reason for the inferior nutritional value. Further tests would be required to verify this assumption. Fortunately, it is economically feasable to add methionine to soybean curd diets. The cost of D,L-methionine is $ .53 per pound feed grade or $ 1.90 per pound food grade (Personal communication, Mr. F. Robertson, Western Brand Products Limited, Edmonton, Alberta). To bring the methionine up to the National Academy of Sciences - National Research Council (United States) level for rats in this test, 0.38 g/100 g diet would be required (Table XIV). The rats consumed an average of 300 g each over the 28 day trial and would, therefore, require an additional 1.14 g methionine. The cost of the additional feed grade D,L-methionine would be .133 ¢ per rat per 28 days. From this it is apparent that the cost of supplementation of soybean curd with methionine would be almost negligible.

The manufacture of synthetic meats from soybean curd was carried out to show the versatility of the curd produced in the present work. In 1966, there were over twenty different variations of synthetic beef, pork, fish and poultry products based on soybean textured protein available in the United States. One problem with these textured products is the cost of the textured protein ingredients, which is over

$ .35 per pound (Altschul, 1965). Another problem is the cost of sophisticated production equipment. The cost of the raw materials in the finished soybean curd produced in this study is approximately $ .09 per pound. The equipment required for the process is simple and relatively inexpensive. This figure could be lowered by carrying out further extractions or sale of the residue.

It has been shown in this study that the use of microorganisms and enzyme systems for the modification and utilization of soybeans, although bringing about desirable changes in texture, results in the formation of undesirable bitter flavors. It has also been shown that the beany and other off-flavors associated with raw soybeans can be effectively removed by proper processing procedures.

It is concluded that a nutritious and acceptable product can be produced from whole soybeans by blending the beans in hot water (80-100 °C), steaming for 30 minutes, straining, precipitating the curd with calcium sulfate, pressing the curd and incorporating flavoring materials and other additives (Patent applied for Schroder and Jackson, June 1969).

In developed countries this protein food base could be made into a host of inexpensive meat substitutes (frankfurters, bologna, sausages, meat spreads, etc.). In the underdeveloped countries, where soybeans can be grown, the products would perhaps overcome the problem of palatability commonly associated with soybeans, thereby, serving to alleviate the ever increasing shortage of dietary protein. The extension of this work to other native sources of protein (oil seeds, leguminous seeds) would appear to be a fruitful and humanitarian endeavour.

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Appendix A

Changes in Bacterial Numbers During Manufacture and Ripening *

		Milk	Cut	Dip	Day 7	14	21	28	35	42	49	56	63
25%	I				2.4×10^5	3.6×10^4	190×10^5	38×10^5	164×10^6	896×10^5	775×10^5	112×10^5	523×10^5
Soybean	II	91×10^5	144×10^6	127×10^6	80×10^5	239×10^5		104×10^5	183×10^5	129×10^5	86×10^5	12×10^5	3.3×10^5
	III	66×10^5	129×10^6	850×10^5	143×10^5	30×10^5		181×10^5	34×10^5	131×10^6	70×10^5	132×10^5	370×10^5
	IV	327×10^5	340×10^6	310×10^6	112×10^6	15×10^4	65×10^4	546×10^4		46×10^4	590×10^4	133×10^5	70×10^4
50%	I				3×10^5	2.7×10^5	320×10^5	27×10^5	350×10^5		279×10^5	370×10^6	287×10^5
Soybean	II	16×10^5	116×10^5	146×10^5	127×10^5	42×10^5		328×10^5	33×10^5	65×10^5	218×10^5	257×10^5	2.8×10^5
	III	1.4×10^5	224×10^6	182×10^6	129×10^5	22×10^4		5.1×10^4	270×10^4	509×10^4	374×10^4	69×10^4	490×10^4
	IV	360×10^5	328×10^6	450×10^6	148×10^6	123×10^5			485×10^4	125×10^6	30×10^5	169×10^5	95×10^5
75%	I				23×10^4	19×10^4	313×10^4	585×10^4	377×10^6		100×10^6	50×10^5	537×10^5
Soybean	II	25×10^5	138×10^5	706×10^5	162×10^5	165×10^4		685×10^4	270×10^4	736×10^4	500×10^4	56×10^4	97×10^4
	III	5.7×10^4	375×10^6	264×10^6	169×10^5	30×10^4		22×10^4	21×10^4	35×10^4	390×10^5	165×10^5	45×10^5
	IV	360×10^5	400×10^6	310×10^6	140×10^6	55×10^5	22×10^4	162×10^4	33×10^4	52×10^4	100×10^6	395×10^6	279×10^6

* Each figure represents the average of two determinations.

Appendix B

Changes in pH During Manufacture and Ripening *

		Milk	Cut	Dip	Day 7	14	21	28	35	42	49	56	63
25%	I	6.72	5.71	5.40	4.75	4.80	4.60	4.50	4.65	4.60	4.60	4.48	4.43
Soybean	II	6.50	5.55	5.31	4.60	4.55	4.42	4.29	4.29	4.15	4.09	4.21	4.28
	III	6.62	5.62	5.30	4.58	4.62	4.58	4.58	4.58	4.31	4.35	4.38	4.45
	IV	6.56	5.12	4.98	4.49	4.41	4.32	4.37	4.39	4.31	4.27	4.21	4.21
50%	I	6.72	5.82	5.32	4.75	4.70	4.50	4.40	4.51	4.19	4.28	4.29	4.18
Soybean	II	6.45	5.40	5.17	4.52	4.45	4.49	4.25	4.29	4.10	4.18	4.20	4.23
	III	6.62	5.48	5.18	4.50	4.49	4.50	4.60	4.44	4.38	4.40	4.51	4.30
	IV	6.72	4.72	4.62	4.40	4.30	4.22	4.20	4.37	4.18	4.11	4.20	4.21
75%	I	6.65	5.50	5.12	4.60	4.55	4.42	4.40	4.30	4.18	4.30	4.19	4.09
Soybean	II	6.42	5.45	5.05	4.47	4.50	4.38	4.32	4.27	4.04	4.00	4.03	4.20
	III	6.55	5.34	5.05	4.50	4.55	4.50	4.53	4.42	4.24	4.21	4.28	4.40
	IV	6.60	4.80	4.62	4.38	4.30	4.20	4.24	4.35	4.18	4.21	4.25	4.40

* Each figure represents the average of two determinations.

Appendix C

Changes in Water Soluble Nitrogen During Ripening *

		0	7	14	21	28	35	42	49	56	63
25%	I	0.16	0.38	0.55		0.87		1.11			1.32
Soybean	II	0.21	0.46		0.75			0.82			1.04
	III	0.28	0.40	0.56	0.96			0.89			1.05
	IV	0.19	0.48		0.72	0.77		0.85	1.01	1.09	1.13
50%	I	0.12	0.29	0.43		0.62		0.86			1.10
Soybean	II	0.14	0.40		0.56			0.78			0.95
	III	0.17	0.31	0.48	0.61			0.69			0.81
	IV	0.14	0.40		0.50	0.61		0.66	0.77		1.01
75%	I	0.13	0.23	0.33		0.63		0.73			0.91
Soybean	II	0.10	0.32		0.52			0.59			0.78
	III	0.16	0.25	0.42	0.49			0.59			0.67
	IV	0.06	0.31		0.32	0.34		0.54	0.58	0.64	0.82

* Each figure represents the average of two determinations.

Appendix D

Moisture Content During Ripening *

		Curd	7	14	21	28	35	42	49	56	63
25%	I	69.00	62.45	64.21	63.16	63.60	61.23	64.78	61.20	65.68	59.25
Soybean	II	76.77	66.79	66.04	66.80	66.88	66.67	66.51	66.67	67.22	66.66
	III	72.32	65.33	64.25	66.00	67.16	66.50	65.64	66.52	67.24	65.00
	IV	76.18	66.82	69.05	68.58	68.46	67.18	67.17	65.09	66.55	66.88
50%	I	71.00	67.86	68.54	69.02	70.42	68.42	69.67	70.55	65.73	66.82
Soybean	II	78.84	69.91	68.75	70.85	70.97	71.57	68.80	68.15	69.42	69.70
	III	76.32	69.57	69.53	72.34	70.70	70.83	70.18	69.97	70.15	71.08
	IV	79.62	67.78	69.08	68.97	67.80	68.15	67.17	65.96	67.56	64.88
75%	I	78.50	72.24	72.86	69.00	72.37	72.51	72.56	71.79	67.36	70.66
Soybean	II	79.38	70.72	71.43	70.83	73.17	71.68		71.48	70.87	71.83
	III	75.96	70.33	70.73	72.35	72.12	72.19	72.13	71.43	71.69	71.32
	IV	85.20	70.56	71.67	71.93	71.20	71.35	70.73	70.45	70.61	70.29

* Each figure represents the average of two determinations.

Appendix E

Nutritional Study Data

	Rat #	Sex	Initial Weight	Total Gain	Avg Daily Gain	Total Feed	Avg Daily Feed	Feed/Gain	PER	Total Feed Wastage
A) Test										
	1	M	57.4	155.2	5.54	360	12.86	2.32	1.93	6
	2	F	41.0	105.7	3.78	298	10.64	2.82	1.59	8
	3	M	47.7	119.0	4.25	279	9.96	2.34	1.91	9
	4	F	43.0	114.0	4.07	314	11.21	2.75	1.63	10
	5	M	44.8	135.1	4.83	325	11.61	2.41	1.86	30
	6	F	53.7	126.6	4.52	343	12.25	2.71	1.66	1
	7	M	56.0	136.0	4.86	332	11.86	2.44	1.83	9
	8	F	56.8	139.9	5.00	349	12.46	2.49	1.80	0
	9	M	61.7	154.3	5.51	358	12.79	2.32	1.93	7
	10	F	57.6	112.0	4.00	305	10.89	2.72	1.65	0
	11	M	42.8	119.1	4.25	294	10.50	2.47	1.82	54
	12	F	44.0	105.7	3.78	300	10.71	2.84	1.58	4
	13	M	47.7	100.8	3.60	284	10.14	2.82	1.59	127
	14	F	45.5	113.4	4.05	299	10.68	2.64	1.70	35
	15	M	48.7	116.2	4.15	282	10.07	2.43	1.84	2
	16	F	39.5	88.4	3.16	247	8.82	2.79	1.60	2
	17	M	47.2	127.9	4.57	296	10.57	2.31	1.94	42
	18	F	57.8	106.3	3.80	304	10.86	2.86	1.57	1
	19	M	45.7	59.2	2.11	203	7.25	3.43	1.30	36
	20	F	42.0	74.9	2.68	237	8.46	3.16	1.42	36
Average Males			49.67	122.3	4.37	301	10.76	2.53	1.80	32.2
Average Females			48.09	108.7	3.88	300	10.70	2.78	1.62	9.7
Overall Average			48.88	115.5	4.13	301	10.73	2.66	1.71	21.0
B) Control										
	1	M	54.2	203.5	7.27	384	13.71	1.89	2.32	0
	2	F	56.0	138.8	4.96	330	11.79	2.38	1.84	0
	3	M	56.6	208.0	7.43	398	14.21	1.91	2.29	0
	4	F	44.9	139.7	4.99	333	11.89	2.38	1.83	0
	5	M	57.2	195.6	6.99	395	14.11	2.02	2.16	21
	6	F	40.1	131.1	4.75	301	10.75	2.26	1.93	12
	7	M	56.5	178.2	6.36	370	13.21	2.08	2.10	0
	8	F	58.2	139.9	5.00	341	12.18	2.44	1.79	0
	9	M	59.8	190.2	6.79	391	13.96	2.06	2.12	0
	10	F	47.9	134.3	4.80	320	11.43	2.38	1.83	0
	11	M	58.5	190.2	6.79	396	14.14	2.08	2.10	1
	12	F	43.5	134.4	4.80	300	10.71	2.23	1.96	0
	13	M	56.1	177.2	6.33	351	12.54	1.98	2.21	0
	14	F	46.8	131.9	4.71	349	12.46	2.65	1.65	7
	15	M	56.3	191.7	6.85	380	13.57	1.98	2.20	0
	16	F	45.9	134.1	4.79	316	11.29	2.36	1.85	2
	17	M	57.1	203.3	7.26	404	14.43	1.99	2.20	0
	18	F	40.0	139.9	5.00	316	11.29	2.26	1.93	0
	19	M	59.3	191.6	6.84	382	13.64	1.99	2.19	0
	20	F	37.4	128.3	4.58	281	10.04	2.19	1.99	0
Average Males			57.16	193.0	6.88	385	13.75	2.00	2.19	1.3
Average Females			46.07	135.4	4.84	319	11.38	2.35	1.86	3.0
Overall Average			51.62	164.2	5.87	352	12.57	2.18	2.02	2.15

Appendix F

Coefficient of Variation (Steel and Torrie, 1960)

$$\text{Coefficient of Variation} \quad CV = \frac{100s}{\bar{x}}$$

where: $$s = \sqrt{\frac{\Sigma X^2 - \frac{(\Sigma X)^2}{n}}{n - 1}}$$

x = each individual protein extimation

n = the number of estimations

$\bar{x}$ = mean of the protein estimations

Appendix G

Sign Test (Steel and Torrie, 1960)

$$\chi^2 = \frac{(|n_1 - n_2| - 1)^2}{n_1 + n_2}$$

where: χ^2 = Chi-square distribution (goodness of fit)

n_1 = number of positives (test less beany than Chinese curd)

n_2 = number of negatives (test more beany than Chinese curd)

Wilcoxon's Signed Rank Test

Table A.18 (Steel and Torrie, 1960)

Appendix H

Analysis of Variance (Steel and Torrie, 1960)

The following is an example of the form of analysis used:

Analysis of Variance for Gain

Correction Term $(CT) = \frac{(\Sigma X)^2}{n}$ where X = all values of gain

n = total number of rats in the test

Total Sum of Squares $= \Sigma X^2 - CT = 66.8$

Sum of Squares for Sex $= \frac{(\Sigma \text{Males})^2 + (\Sigma \text{Females})^2}{20} - CT = 16$

Sum of Squares for Diet $= \frac{(\Sigma \text{Control})^2 + (\Sigma \text{Test})^2}{20} - CT = 30.1$

Sum of Squares for Interaction between Sex and Diet

$$= \frac{(\text{Sum of Male Control})^2 + (\text{Sum of Female Control})^2 + (\text{Sum of Male Test})^2 + (\text{Sum of Female Test})^2}{10} - CT$$

$$- (\text{SS for sex} + \text{SS for diet}) = 6$$

Analysis of Variance Table

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F Value
Sex	16	(levels sex-1)=1	16	39.0 ***
Diet	30.1	(levels diet-1)=1	30.1	73.4 ***
Sex x Diet	6	1	6	14.63***
Error (by difference)	14.7	36	0.41	
Total	66.8	(n-1) = 39		

where:

$$\text{mean square} = \frac{\text{sum of squares}}{\text{degrees of freedom}}$$

$$\text{F value} = \frac{\text{treatment mean square}}{\text{error mean square}}$$

	5%	1%	0.5%
Levels of significance (Table A.6 Steel and Torrie)	4.08*	7.31**	8.83***